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ASSESSMENT OF THE POST TREATMENT FUNCTIONAL OUTCOME IN PATIENTS OF OSTEOARTHRITIS KNEE MANAGED WITH INTRA ARTICULAR OZONE AND PRP COMBINATION THERAPY

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INTRODUCTION

Osteoarthritis (OA) is a very common joint disease, which is characterised by progressive loss of articular cartilage, subchondral bony sclerosis, changes in the synovial membrane and reduced viscosity of the synovial fluid. The most commonly affected joint is the knee, and the rate of knee OA has been reported as 30 % in subjects over 50 years of age examined by radiographic imaging.

There is no definitive treatment method to prevent progression of OA. However, a number of treatment methods, including modification of daily routine, medical management, physiotherapy, intraarticular injections and arthroplasty, have the primary aim of pain relief and increasing joint functions. The most appropriate treatment choice for the patient depends on the patient's clinical history, contraindications to specific treatments and how well the patient would be able to tolerate the treatment being considered. Especially in cases where the target patient group is of advanced age and first line treatment methods have not been successful, doctors have increasingly preferred injections because of the potential side effects of non- steroidal anti-inflammatory drugs (NSAIDs). Topical medications are often used for short-term relief, but are not adequate in cases of severe osteoarthritis.

The knee joint cartilage is non-vascular. Since nourishment is dependent on diffusion, as intra-articular injections are given at high concentrations, they have become the preferred method in cartilage regeneration. Various intra-articular agents have been developed for this purpose [16, 24, 26].

Recently, Platelet Rich Plasma has been used extensively in orthopaedic disorders for many reasons, including its autologous nature, higher efficacy, and few adverse effects [5,6]. The PRP procedure includes administration of platelets derived from the patient's blood and growth factors derived from these platelets to the pathological area [5,6]. Platelets are concentrated by plasmapheresis and anti-coagulated blood is segregated into its' solid and liquid components by centrifugation. In the last phase, plasma and platelets are separated from red cells and white cells. Platelet- rich-plasma includes white blood cells and some proteins as well as platelets [7]. Neutrophils and monocytes may induce localised anti-inflammatory effects, which can accelerate tissue regeneration and repair.

Tissue re-generation is a complex process consisting of several phases such as chemotaxis, angiogenesis, cell proliferation, and matrix formation. Platelets promote these phases by the release of growth factors such as platelet- derived growth factor, vascular endothelial growth factor (VEGF), endothelial cell growth factor and fibroblast growth factor. Besides, transforming growth factor beta (TGF-β), which is a protein also released by the platelets, may demonstrate inhibitory effects on tissue regeneration and cause unpredictable results [8]. Using PRP treatment, disease progression can be controlled and a positive impact on healing of the affected joint can be obtained [7].

Ozone injection is another method used for injection. The effects of ozone were discovered in mid-19th century. Ozone is an unstable molecule formed by the combination of oxygen atoms. The therapeutic effects of ozone include anti-inflammatory effects, positive impact on cellular metabolism following increased use of glucose, improvement of protein metabolism, conversion of unsaturated fatty acids into water-soluble compounds and improvement of erythrocyte activity [9]. There are publications supporting the use of ozone injection in knee OA treatment [10]. Ozone gas combined with oxygen gas is administered to the pathological area. Oxygen-ozone injection improves tissue oxygenation and inhibits inflammatory mediators such as TNF alpha. Ozone can also induce moderate oxidative stress and inhibit inflammatory responses. Ozone also has moderate analgesic effects through phosphodiesterase A2 blockage [11]. Several trials provided results confirming that ozone injection was safely used in treatment of lumbosacral disc herniation, post-laminectomy syndrome, and shoulder disorders [12,13]. Moreover, ozone treatment was shown to be an efficient treatment method for managing symptoms after traumatic meniscal injuries [14].

Both PRP and Ozone have recently gained interest in the treatment of knee OA. Although similar in efficacy, ozone is also used for PRP activation. Currently there are not many studies comparing efficacy between PRP and PRP + ozone treatments. In the published literature, there's only one similar retrospective study conducted by Sabanci et al. [15] which evaluated the efficacy of PRP treatment combined with ozone. However Sabanci et al. [15] did not compare PRP treatment alone to combination treatment. Instead, they com- pared different doses of combination treatments. This is one of the first studies in India , conducted in the Indian population, in which the objective was to evaluate the post therapy functional outcome in patients diagnosed with knee OA, who received a novel PRP and Ozone intra-articular combination therapy for Osteoarthritis of the knee.

Osteoarthritis Of The Knee

OA is a degenerative condition that produces pain, decreases function and affects the quality of life significantly. OA has great morbidity and low mortality which makes it a chronic disease especially among the older population. (Battle 2002).

The evolution of knee OA is slow and progressive (Battle 2002; Mishra 2011). Although symptoms can diminish, radiological signs can rarely get better (Dougados, 1992). The radiological worsening over time occurs in 33-66% of patients. Episodes of pain lasting from days to weeks are a regular clinical presentation. If pain persists for months, subchondral bone collapse should be suspected. An acute onset of pain could indicate necrosis of femoral bone (Dougados 1992).

OA affects mainly middle aged patients. In the younger age group both sexes are equally affected but later (50 years +) females are mainly affected. Obesity, familial history, high body mass index (BMI) and repeated trauma are the precipitating factors to develop OA (Mishra 2011). In that context, OA is not merely a mechanical problem, but a chronic inflammatory condition with cell and biochemical alterations, that justify pharmacological and ozone therapy (De Lucas 2005; Samper-Bernal 2013; Vaillant 2013; Borrelli 2015).

In knee OA the main pathological changes are progressive loss of cartilage, meniscus and capsule of the joint (Mishra 2011). Structural changes on knee OA are visible on standard X-ray and include narrowing of the joint space (due to cartilage loss), formation of osteophytes at the joint margins, and bony sclerosis (increased density or thickness of the bone) just underneath the articular cartilage (Goldring 2007). (Figure 1)

Figure: Severe Knee OA and radiological changes. In this left knee radiography, there is destruction of articular cartilage, narrowing of articular space, sclerosis of subchondral bone, peripheral osteophyte formation and subchondral cysts.

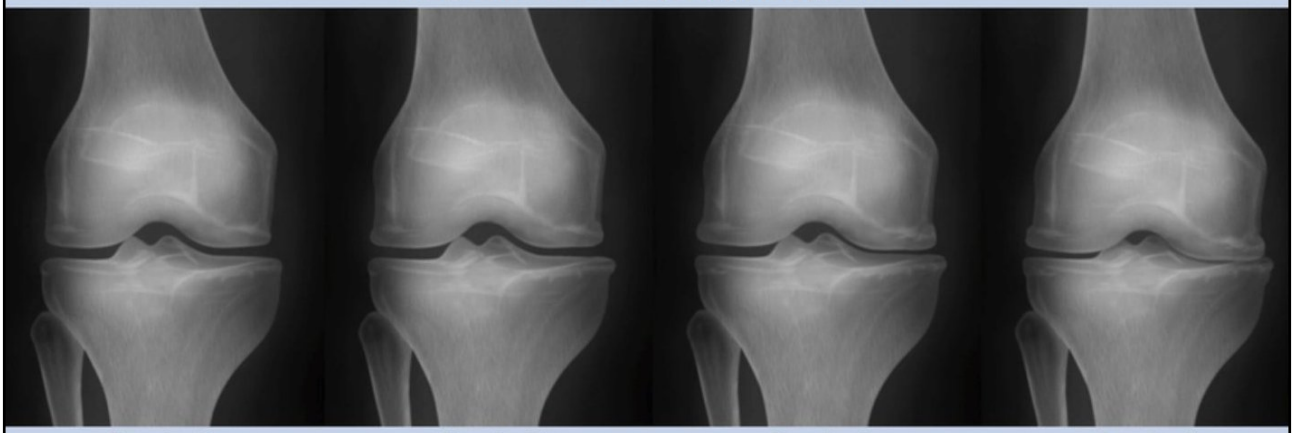
The cytokines released by chondrocytes implicated in the catabolic process of the extra cellular cartilage matrix are: IL1, IL6, IL8, IL17, LIF, TNF-α, IFN-γ. All of these cytokines produce cartilage damage (Battle 2002). The proteolytic degradation of extra cellular matrix forms an important mechanism of articular degradation. In such a case, the future therapy in OA should produce an inhibition of proteolytic enzymes (MMP or mineral metalloproteinases), nitric oxide synthesis (NOs), pro-inflammatory cytokines (IL1, IL6, TNF-α), and apoptosis through caspases. On the other hand, OA management should favour anti-inflammatory cytokines (IL4, IL10, IL13), and stimulate growth factors (TGF-β, IGF-1) (Battle 2002).

In OA there is augmentation of oxygen free radicals. They inhibit collagen and proteoglycans, accelerating the cartilage matrix disintegration, and narrowing the joint space (Yu 2010). IL-1, which depends of the production of ROS (Reactive Oxygen Species), is implicated in the DNA damage to chondrocytes (Davies 2008).

The surgical cell therapy goal is to transplant chondrocytes and stem cells in order to recover vascularisation and trophism of bone and cartilage (Battle 2002).

The last option for advanced knee OA is the surgical treatment, in order to replace the damaged joint. Knee prosthesis is a costly and invasive surgical procedure not free of side effects and complications (Fernández-Cuadros 2013, Borreli 2015).

Kellgren-Lawrence (KL) grading scale



	Grade 1	Grade 2	Grade 3	Grade 4
CLASSIFICATION	Normal	Doubtful	Mild	Moderate	Severe
DESCRIPTION	No features of OA	Minute osteophyte: doubtful significance	Definite osteophyte: normal joint space	Moderate joint space reduction	Joint space greatly reduced: subchondral sclerosis

Figure : Kellegren-Lawrence grading for Osteoarthritis Knee.

Ozone Therapy

Ozone is the third most potent oxidant agent, after fluorine and persulphate, which explains its high reactivity (Bocci 2011).

Ozone is the allotropic or unstable form of oxygen (Iliakis 2001, Paolini 2009, Seyman 2012). It is used to treat several autoimmune, degenerative, infectious and musculoskeletal diseases (Mawsouf 2011). It is postulated that Ozone has analgesic, anti-inflammatory, immunomodulatory and trophic properties (Paoloni 2009).

Ozone decomposes spontaneously and therefore it is not storable. The ozone concentration is halved at 30ºC within 25 minutes, at 20º within 40 minutes and at minus 50ºC within 3 months (Bocci 2011). Ozone decomposes rapidly, splitting in O2 and O- (monatomic O) which is very reactive, and it has anti infectious, antiviral, anti-parasitic, anti-fungal and antitoxic properties (Madrigal 2007). The Ozone disintegration releases 24,27kcal producing an important peripheral vasodilator effect (Madrigal 2007).

Ozone properties have a doses/effect ratio (Bocci 2006; Bocci 2011). Most medical ozone generators deliver ozone concentrations from 1 to up to 70-100ug/ml (Figure 2). The total ozone dose is equivalent to the gas volume (ml) times the ozone concentration (ug/ml). For different medical uses, the correct ozone doses must be known (Bocci 2011). However, the therapeutic spectrum is between 10-80 ug/ml (Madrigal 2007).

Figure 2: Ozonotherapy equipment (Ozonosan® alphaplus). Dose of 20 µg/ml.

Figure 3: Ozonotherapy equipment (Ozonics). Dose of 10 µg/ml.

Ozone dissolved in the plasmatic H2O reacts readily with a number of biomolecules and disappears. There are two compounds (reactive oxygen species or ROS and lipid oxidative products or LOPS) which represent the "ozone messengers" and are responsible for the biological and therapeutic effects. ROS are produced rapidly and immediately, in the early phase (mainly Hydrogen peroxide or H2O2) and are responsible for the early biological effects on blood (erythrocytes, leucocytes, platelets). On the contrary, LOS, which are simultaneously produced have a far longer half-life, they reach the vascular system and interact with several organs, where they trigger late effects. Some of these real targets are liver (in chronic hepatitis), vascular system (in vasculopathies), while other organs are probably involved in restoring normal homeostasis (central nervous system, gastrointestinal tract, mucosal associated lymphoid tissue) (Bocci 2006, Mawsouf 2010, Bocci 2011).

The biological effects of Ozone elicited during exposure to plasma are due to the formation of ROS and LOPs (Ozone messengers). ROS improves O2 delivery in erythrocytes. They stimulate leucocytes and immune activation. On platelets Ozone favours the release of autacoids (local hormones) and growth factors. LOPs increase the release of NO- on the endothelium. They favour the generation of super gifted erythrocytes and release of stem cells on the bone marrow. Finally, LOPs acts over other organs, activating the upregulation of Oxidative Shock Proteins (OSP) and antioxidant enzymes (Superoxide dismutase, catalase and glutathione-peroxidase). (Table 1) (Bocci 2006; Bocci 2011).

Subtrates	Messengers	Target organs	Functional modificactions
Ozone in plasma	ROS	• Erythrocytes • Leucocytes • Platelets	Improved O2 delivery. Inmune activation. Release of autacoids and growth factors.
	LOPs	• Endotelium • Bone marrow • Otherorgans	Increased release of NO-, generation of erythrocytes. Release of stem cells. Upregulation of Oxidative Shock Proteins and antioxidant enzymes (Ozone tolerance).

Table: Effect of Ozone on target organs and functional modifications (Bocci 2011).

The antioxidant system has evolved during the last two billion years as an essential defense against Oxygen. Antioxidant system is made up of scavenger components: albumin, Vitamins C and E, uric acid, bilirrubin, cysteine, ubiquinol, alpha lipoic acids and intracellular antioxidants (superoxide dismutase, catalase and glutathione peroxidase), protein (ceruloplasmin, which is able to chelate free iron and copper that otherwise, could favour the formation of hydroxyl radicals). In this context, a small amount of Ozone exposed to blood can undergo a transitory oxidative stress absolutely necessary to activate several biological systems and functions without detrimental or deleterious effects (Bocci 2011).

The purpose of Ozone is to produce acute oxidative stress. This stress must be adequate (otherwise it is a placebo), calculated (neither below nor too much above threshold levels) and transitory (not chronic oxidative stress). It is of paramount importance neither to override the antioxidant system nor to cause any toxicity. The upregulation of the antioxidant system is followed by repeated but small oxidative shocks (Bocci, 2011) (Table 2).

Ozone is an effective modulating agent for biological oxidative stress. It stabilises pro-oxidant and antioxidant systems and also inflammation (Madrigal 2007).

The following situations limit the use of ozone: a) significant deficit of glucose-6-phosphate dehydrogenase (favism is an haemolytic disease in people lacking that enzyme); b) pregnancy in the early phase (to exclude any mutagenic risk, although unlikely); c) patients treated with angiotensin converting enzyme inhibitors; d) hyperthyroidism, thrombocytopenia, serious cardiovascular instability; and e) allergy to Ozone (Bocci 2011). Except for these situations, Ozone is very safe and it has no side effects, then no formal contraindication is stated by most researchers (Madrigal 2007).

There are several pathologies such as arteriosclerosis, diabetes, ischemia, osteoarthritis, neurodegeneration, nephropathies, chronic viral infections, autoimmune diseases and cancer; where there is an imbalance between oxidants and antioxidants, leading to chronic oxidative burden and death (Bocci 2006, Bocci 2011).

Ozone is a wonder drug because it can produce four extraordinary phenomena: 1) the induction of oxidative shock proteins (OSP); 2) the upregulation of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase); 3) the reduction and/or normalization of oxidative stress; and 4) the probable release of bone marrow stem cells (Bocci 2006, Madrigal 2007, Bocci 2011).

Ozone mobilises bone marrow stem cells (BMSC) and produces LOPs, which induce NO synthase (NOs). This produces inhibition of platelet-leukocyte aggregation. Besides, NO produces neovascularisation and neoangiogenesis. NO activates MMP-9 (matrix metalloproteinase 9) indispensable for stem cell mobilisation (Bocci 2006, Bocci 2011).

As a resume, Ozone is a molecule able to act simultaneously on several blood components. Ozone produces messengers such as ROS and LOPs; they can act locally or systemically in practically all cells of an organism. Four decades of clinical experience have shown that ozone can act as a disinfectant, an oxygen donor, an immunomodulatory, a paradoxical inducer of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase), a metabolic enhancer, an inducer of endothelial NO syntheses, and possibly an activator of stem cells with consequent neovascularisation and tissue reconstruction (Bocci 2006, Bocci 2011).

Ozone In Orthopaedic Diseases

The first author who had the idea to inject small volumes of ozone in patients affected by tendinitis and myofascial pain was Dr. Alexander Balkany in Zurich in the 1970s. After him Riva Sanseverino (1989), Verga (1989), Siemsen (1995) have begun to treat acute and chronic polyarthritis (OA of the knee, hip, interphalangeal joints, sacroiliac joints, etc.), epicondylitis and carpal tunnel syndrome with intra articular or periarticular insufflation of small volumes of O2-O3 or Oxygen-Ozone (5-10 ml in one or three sites with an ozone concentration from 5 to 15ug/ml) with very encouraging results. In Morton's disease (neuroma), up to six infiltrations of gas (4ml each at 20ug/ml) have yielded great pain relief. In a very informative review, Siemsen (1995) reported that application of medical ozone in acute and chronic painful joint diseases is a complementary method of treatment to obtain rapid pain relief, decongestion, disappear of edema, reduction of local temperature and increase mobility. If performed by an expert orthopaedic surgeon, the treatment is not risky and causes only transitory local pain that disappears in 5-10 minutes without any other adverse effect (Siemsen 1995).

The pathophysiology of OA diseases is complex and characterized by the softening and even destruction of articular cartilage, with increased matrix degradation due to collagenases and proteoglycanases. The enzymes may be secreted by activated chondrocytes and monocytes, which by releasing IL-1 and TNF-α amplify the inflammation. Synthesis of PGs increases several fold and there is a natural attempt to maintain a biomechanical adequate matrix. In contrast to Rheumatic Arthritis, pannus does not develop. Joint pain may be aggravated by concomitant synovitis (Bocci 2011).

Drug therapy for OA is symptomatic, aiming to reduce pain and disability. Inhibitors of cyclooxygenase I (COX I) are in wide use, with some side effects, and are being substituted with inhibitors of cyclooxygenase II (COX II), but less successfully. Local injection of glucocorticoids into a given joint can be carried out no more than twice per year (Bocci 2011).

Conventional medicine does not have a cure for OA. Patients search for complementary therapies. By now, innumerable patients have been treated with ozone, and they have experienced great pain relief for a long time. It is surprising that ozone, a potent oxidant, injected into the synovial space does not elicit further inflammation nor degeneration, but it is believed to produce just the opposite paradoxical effect (Bocci, 2011). It is believed that Ozone injected on the synovial fluid generates ROS and LOPs, which are responsible for the positive effects of Ozone therapy: a) possible inactivation-inhibition of release of proteolytic enzymes/proinflammatory cytokines; b) stimulation of proliferation of chondrocytes (probably via H2O2- ROS) and fibroblasts, with increased synthesis of matrix and possibly of articular cartilage. The induction of the synthesis of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) are a crucial event in the adaptive response to chronic oxidant state to Ozone. That is the reason why Ozone must be injected at low doses at the beginning; c) probable inhibition of release of bradykinin/synthesis of inflammatory PGs with reabsorption of edema and pain relief; d) an increased release of IL-1 soluble receptor or of some other soluble receptors and antagonists able to neutralize pro-inflammatory cytokines such as IL-1, IL-8, IL-12, IL-15 and TNF-α; e) conversely, the release of immunosuppressive cytokines (TGF-β1 and IL-10) may inhibit inflammation (Iliakis 2001, Paolini 2009). Among several growth factors, TGF-β1 is interesting because it modulates the expression of integrins and stimulates the synthesis of matrix proteins such as collagen and glycosaminoglycan's (Trippel 1995; Qi and Scully 1997; Grimand 2002, Jani 2012).

In 1988, Verga noted pain relief after infiltrating trigger points in myalgia with O2-O3 and proposed to use an indirect technique by injecting the gas into localizable points in the paravertebral muscle (locus dolente) corresponding to the metamere of the herniated disc. Now this is called the indirect approach or chemical acupuncture (named by Bocci since 1988) to clarify that the beneficial results is obtained via the chemical reaction to Ozone (Bocci 2011).

It is hypothesised that the formation of Ozone messengers ROS and LOPs would act in the synovial fluids in two phases. In the first phase Ozone would inhibit inflammation diminishing pro-inflammatory cytokines (like PGE2 and leukotrienes via decreasing phospholipase A2, COX I and COX II inhibition) diminishing kallikrein and bradykinin. It could inhibit the release of serotonin and MPs or metalloproteinases such as collagenase, gelatinase and aggrecanase, avoiding cartilage degradation (Bocci 2011, Buric 2014). On the late phase, Ozone would act over the upregulation of antioxidant enzymes, OSP (HO- 1) inhibitory cytokines (IL-4, IL-10, TGF-β), neoangiogenesis, NO syntheses and release of endorphins, ACTH and cortisol. All of these would act in the reparative process in the articular joint by stimulating chondrocytes, fibroblasts and stem cells to synthesize proteoglycans, glycosaminoglycans and collagen (Bocci 2011).

The explanation for that efficacy is that O2-O3 acts on several cytokines which are lately responsible for the degradation and the repair of cartilage and bone in OA. Ozone stimulates the production of TGF-β1 which accelerates the cell recovery (Madrigal 2007).Ozone induces the production of cytokines: IFN-γ (Bocci y Paulesu 1990, Madrigal 2007, Bocci 2011), IFN-β, IL-2, IL6, IL-8, TNF-α, TGF-β (Paulesu 1991, Bocci 1998, Larini 2001, Madrigal 2007, Bocci 2011), IL-4 and IL-12 (Madrigal 2007).(Table 3)

Cytokine/ Cell Target	Ozone effect	Article Review
MMPs inhibition	↑	Bocci 2006, Bocci 2011, Buric 2014
NO synthesis	↑	Bocci 2006, Bocci 2011, Borrelli 2015
PGE2 inhibition	↑	Borrelli 2015
Inhibition of Pro-inflammatory Cytokines:		
• IL-1	↑	Bocci 1993, Iliakis 2001, Chang 2005, Jani 2012
• TNF-α	↑	Larini 2001, Madrigal 2007, Borrelli 2015, Chang 2005
• IFN-γ, IFN-β	↑	Bocci y Paulesu 1990, Larini 2001, Bocci 2006, Madrigal 2007, Bocci 2011, Jani 2012, Borrelli 2015
To stimulate anti-inflammatory cytokines:		
• IL4, IL6, IL10, IL13	↑	Bocci 1993, Iliakis 2001, Larini 2001, Chang 2005, Madrigal 2007, Borrelli 2015
Growth Factors:		
• TGF-β, IGF-1	↑	Paulesu 1991, Bocci 1993, Trippel 1995, Qi 1997, Larini 2001, Grimand 2002, Bocci 2005, Madrigal 2007, Fuccio 2009, Bocci 2011, Jani 2012, Borrelli 2015
Cell Therapy:		
• Chondrocytes, stem cells	↑	Bocci 2006, Bocci 2011

Table 3: Ozone therapeutic targets in knee OA.

It would be interesting to examine synovial fluid before and after Ozone therapy, because there are no experimental studies on the mechanism of action of Ozone in knee OA (Borreli 2015, Hashemi 2015).Unfortunately this is not possible because it is difficult to collect patients and most of them are treated privately. However, Chang has recently demonstrated that Ozone in synovial fluid diminishes the production of the proinflammatory cytokines IL-6, IL-1β and TNF-α (Chang 2005).

On the other hand, the private industry is no interested in the study of the efficiency of Ozone on pain in knee OA for economic interests. That is the reason why there are only small series of case-reports worldwide. It is of paramount importance to originate and reproduce new and interesting clinical studies that sustain the effectiveness of Ozone, so it could become useful for the benefit of both science and patients.

Ozone Infiltration Technique And Effectiveness In Osteoarthritis Of The Knee

O2-O3 in osteoarticular diseases produces: a) better vascularisation on bones and cartilage, accelerating anabolism and recovery; b) anti-inflammatory effect by ozone activation over PGs (prostaglandins); c) immunomodulatory effect on autoimmune and inflammatory diseases (such as Rheumatoid Arthritis and Osteoarthritis); d) trophic effects on bone and cartilage (Madrigal 2007).

In knee OA there is great variability in terms of side of injection, volume and gas concentration of Ozone therapy (Borreli 2015). The ozone treatment on knee joint could be intra articular, periarticular and subcutaneous. The O2-O3 can be applied twice/week for 5-6 weeks. The concentration of O2-O3 must be 10ug/ml, the volume of the gas should be 20ml for intra articular injections, 10ml for periarticular and subcutaneous injections (Figure 3). The treatment is painless if the injections are applied slowly (Madrigal 2007).

Figure 3: Intra articular infiltration of 10 ml of Ozone.

After the first ozone therapy treatment, some other treatments can be applied afterwards (1-2 per year) for maintenance. It all depends on the effect over the articular physiology of the knee. It is accepted that ozone improves the range of movement (ROM) in Osteoarthritis of the knee (Madrigal 2007).

On knee OA, ozone slows the degenerative process and improves the ROM. Besides, ozone improves the cell permeability and diminishes articular effusion (Madrigal 2007).

The published studies on the use of Ozone in knee OA are limited. Fahmy and Riva –Sanseverino were the first to use Ozone in the treatment of Knee OA and in rheumatoid arthritis (Fahmy 1981, Riva-Sanseverino 1989). Escarpenter reported the treatment of 123 patients with knee OA (Escarpenter 1997). Riva-Sanseverino treated 83 knee OA patients with amelioration of pain (Riva Sanseverino 2002). Milanés in 321 patients diminished pain and improved articular function (81% of success) (Milanés 2009). Baeza-Noci has reported 80% success in knee and hip OA, while de Lucas published 74% in Knee OA (de Lucas 2005, Baeza-Noci 2011). More recently, Cabot found 82-87% success in 57 knee OA/chondromalacia patients after ozone treatment (Cabot 2012). (Table 4)

Clinical variable	**Ozone effect**	**Article Review**
Pain	↓	Fahmy 1981, Riva-Sanseverino 1989, Siemsen 1995, Escarpenter 1997, Riva Sanseverino 2002, Muto 2004, Benvenuti 2006, Milanes 2009, Paolini 2009, Moretti 2010, Mishra 2011, Cabot 2012, Calunga 2012, Li 2013, Samper 2013, Hasehmi 2015
Function	↑	Benvenuti 2006, Milanes 2009, Yu 2010, Mishra 2011, Cabot 2012, Li 2013, Samper 2013, Hasehmi 2015
Rigidity	↓	Samper 2013, Camelia 2014,
Inflammation	↓	Siemsen 1995, Benvenuti 2006, Madrigal 2007, Yu 2010, Valliant 2013,

Platelet Rich Plasma - Role in musculoskeletal tissues

PRP has been proposed as a promising biologic treatment with a wide ranging applications in musculoskeletal disorders (Lansdown and Fortier 2017). Injury type is significant, with benefits shown in the treatment of patella tendinopathy (Dragoo et al. 2014) and OA (Laudy et al. 2015) but not in Achilles tendinopathy or hamstring injuries (Manduca and Straub 2018). The available clinical studies on PRP as a treatment option suggest a good potential in favouring pain reduction and improved function for articular injuries to the ankle, knee and hip (Engebretsen et al. 2010). The evidence base for PRP across different injury types has been criticised for being inconsistent and uncertain (McNamee et al. 2018). In a meta-analysis, Grassi et al. (2018) advocated PRP treatment as a safe procedure with negligible adverse effects that is readily available and has a minimal risk of reactivity compared to other exogenous compounds owing to the autologous nature of PRP injections. However, existing large and indiscriminate use of PRP injections for the treatment of acute muscle injuries in clinical practice is not justified by evidence (Grassi et al. 2018). Thus, caution should be applied to use in knee OA.

In muscle strain and tendon injuries, no statistical or clinically significant differences were found for RTP duration and re-injury rate, leading to the conclusion that PRP is no more effective than a placebo injection or intensive rehabilitation (de Vos et al. 2010, 2014; Creaney et al. 2011; de Jonge et al. 2011; Thanasas et al. 2011; Krogh et al. 2013; Hamilton et al. 2015; Reurink et al. 2015; Liddle and Rodríguez-Merchán 2015); however, other studies have pointed to enhanced recovery (A Hamid et al. 2014; Chen et al. 2018). Between these reports, significant differences in the quality of the study are noted and should be used as a guide when analysing knee OA efficacy.

In knee OA, PRP injections aim to stimulate cartilage repair and offer relief to other osteoarthritic symptoms, potentially delaying the need for joint replacement surgery. PRP injections have shown to influence the entire joint environment, leading to a short-term clinical improvement (Filardo et al. 2012a, b) with PRP injections being considered a safe procedure with more favourable outcomes when compared to alternative treatments (Laver et al. 2017). PRP is relatively easy to use due to its simple and rapid preparation and the minimally invasive administration requiring a simple intra-articular injection. Adverse effects are likely to be reduced due to the patient's own protein use and bioactive molecules can be concentrated to achieve the desired dosage, also eliminating potential drug interactions (Zhu et al. 2013). Without a synthetic element, PRP is, for the most part, not considered to be a drug or therapeutic substance and does not therefore need to fulfil the regulatory requirements needed for other biologic therapies.

Platelet Rich Plasma - Use in Knee Osteoarthritis

The use of PRP in the treatment of degenerative knee OA has increased in recent years given its apparent high margin of safety and ease of production and administration (Smith 2016). Contrasting scientific evidence exists regarding PRP injections for knee OA, with the efficacy of PRP injections widely reported (Rahimzadeh et al. 2018). The enhanced effectiveness of PRP for pain treatment and knee joint function in comparison to HA or placebo and positive outcomes in all stages of knee OA (early, middle and late), have all been reported (Kanchanatawan et al. 2016; Dai et al. 2017; Cole et al. 2017). In addition, the effects of PRP seemingly last longer and are superior in comparison with intramuscular injection therapies (Prieto-Alhambra et al. 2014). Comparisons between intra-articular injection of PRP and placebo and HA therapy in mild and moderate knee OA have generally shown higher clinical outcome scores with PRP use (Filardo et al. 2012a, b). Similarly, using meta-analysis to compare the efficacy of PRP injections against placebo or other therapeutic means for the treatment of knee OA (Bennell et al. 2017) has reported greater pain reduction (Laudy et al. 2015) and functional improvement (Chang et al. 2014) with the use of PRP. However, this is at the expense of an increase in nonspecific adverse events (Khoshbin et al. 2013).

PRP use has been advocated as a treatment option in all stages of knee OA. Intra-articular PRP injections in active patients with knee OA show significant improvements in pain reduction, improved symptoms and QoL (Gobbi et al. 2012). This could be due to the immediate and sustained release of growth factors over a prolonged period, which enhances healing resulting in sustained clinical effects (Dhillon et al. 2017). Symptomatic relief for up to 12 months with increased benefits to patients with early knee degenerative changes has been found (Campbell et al. 2015) with significant improvements in function and reductions in pain with three injections per month yielding significantly better outcomes in the short-term (Huang et al. 2017). Improved pain outcomes after 3 months with a greater effect in lower OA grades have been reported (Montañez-Heredia et al. 2016). In moderate knee OA, functional status and pain have improved with a minimum of two injections (Kavadar et al. 2015). In late-stage knee OA, it may be that only a single PRP intra-articular injection is required to provide effective pain relief, thus improving activities of daily living and QoL (Joshi Jubert et al. 2017).

Scientific Study into the efficacy of PRP has been focused on drawing the comparison between the effects of I/A PRP injections and other injection therapies. In multiple studies, PRP injections have improved outcomes in comparison to HA and placebo controls & appear more efficacious in reducing symptoms and improving quality of life. (Raeissadat et al. 2015; Kanchanatawan et al. 2016). Kon et al. (2011) examined three homogenous groups of patients treated with three injections of PRP, low molecular weight HA and high molecular weight HA and concluded that autologous PRP injections have longer efficacy than HA injections and enhance articular function. The results showed improved outcomes for the PRP group at 6 months with younger and more active patients achieving better results with a low degree of cartilage degeneration (Meheux et al. 2016). Conversely, PRP causes a significantly greater acute inflammatory response and an increase in synoviocyte cell death (Braun et al. 2014) and induces more transient reactions than HA (Riboh et al. 2016). Spaková et al. (2012) compared three PRP injections with three HA injections in a randomised controlled trial (RCT) on 120 patients and discovered better Western Ontario and

McMaster Universities Arthritis Index (WOMAC) scores and Numerical Rating Scale (NRS) in the PRP group compared to the HA group (Table 3). In a separate RCT with 120 patients, Cerza et al. (2012) compared four PRP injections at 1-week intervals with low molecular weight HA and observed better improvement of WOMAC scores at 24 weeks in the PRP group. No correlations with the grade of OA were found in either study. Additionally, better WOMAC scores were achieved at 24 weeks using PRP by Sánchez et al. (2012) who examined 126 patients in a RCT with different grades of OA and compared three PRP injections at 1-week intervals with HA. Similarly, better outcomes have been documented when comparing PRP to HA groups at 6 months (Li et al. 2011; Say et al. 2013). Patel et al. (2013) compared normal saline with PRP and demonstrated that PRP significantly improved WOMAC scores following 6 months in comparison to the placebo groups, with patients experiencing benefits as early as 18 days. In a follow-up study, Patel and Dhillon (2014) hypothesised that the anti-inflammatory effect and chondral remodelling induced by PRP could be the reason for the improved clinical effects.

AIM

1. To assess efficacy and clinical outcome of Ozone and PRP injection (combination therapy) in patients with primary knee osteoarthritis.

OBJECTIVES

1. To establish the efficacy of the protocol for intra articular ozone and PRP combination therapy in treating primary knee osteoarthritis; primarily in terms of pain relief, morbidity reduction and functional improvement.
2. To asses the co-relation between the post therapy functional outcome in correlation to the Kellegren- Lawrence grade of the affected knee at presentation.

REVIEW OF LITERATURE

In the 2019 study conducted by Bahar Dernek and Fatma Nur Kesiktas comparing the efficacy of combined ozone and platelet-rich-plasma treatment versus platelet-rich-plasma treatment alone in early stage knee osteoarthritis, similar efficacy was observed between treatment with PRP alone and treatment with PRP in com- bination with ozone. However, patients receiving ozone treatment are less likely to experience post-injection pain and are more likely to recover faster when compared to patients receiving PRP treatment alone. In both PRP alone and combined treatment groups, post-treatment VAS and WOMAC scores at month 1, month 3, and month 6 showed a significant reduction compared to pre-treatment scores ($p < 0.001$). Physical function and total WOMAC scores as well as VAS scores at post-treatment month 3 were significantly lower in the combined treatment group compared to the PRP alone group. Moreover, in the combined treatment group, VAS scores on Day 10 and hyper-inflammation at the injection site was significantly lower than the PRP alone group. [1]

In the 2022 study conducted by Puxidan Huang, Rui Wang, Xiaolin Pang, Yongtao Yang, Yuan Guan, Dongya Zhang on platelet-rich plasma combined with ozone prevents cartilage destruction and improves weight-bearing asymmetry in a surgery-induced osteoarthritis rabbit model, PRP combined with O3 may prevent cartilage destruction and improve weight-bearing asymmetry by restoring homeostasis between anabolism and catabolism of extracellular matrix in progressive OA. Furthermore, a combination of PRP and O3 might achieve even better results than the two agents alone. [2]

In the 2018 study conducted by Lale Yeprem, Aysegul Ellialtioglu, Jose Baeza-Noci, the efficacy of intra-articular PRP, Ozone and Ozone+PRP injections in patients with osteoarthritis 120 patients were diagnosed of OA according to the criteria of the American College of Rheumatology and included into this study. Patients were randomly divided into three equal groups (40 patients each group). We include patients whose VAS score was 5 or above. Patients with inflammatory, endocrine and metabolic disturbances, and patients who had meniscectomy within the past 10 years, extra articular surgery within the last year, arthrocentesis in last 6 months or any drug given intra-articular were not included. We did not include either patients with misalignment.

Patients in the first group were treated with intra-articular injections of ozone/ oxygen gas mixture 2 times/week, at 10 μg/mL concentration and a volume of 5 mL for a total of 12 injections. Patients in the second group were treated only with PRP injections once a week for a total of 3 times. Patients in the third group were treated with intra-articular injections of Ozone+PRP (10 μg/ml concentration, 5 ml volume) once a week for a total of 3 times. The pain levels of patients were measured with Visual Analog Scale (VAS). Side effects were collected using an open list record. All

the groups improved their baseline VAS in a significant way, although the group with the best results was group 3 with the combination of ozone and PRP in the same injection. Further studies are being conducted on the same by the authors to further the understanding on this. [3]

In the 2015 study conducted by Tahir Mutlu Duymus, Serhat Mutlu, Bahar Dernek · Baran Komur, Suavi Aydogmus, Fatma Nur Kesiktas, The purpose was to compare the efficacy of treatment in three groups of patients with knee osteoarthritis (OA) given an intra-articular injection of platelet-rich plasma (PRP), hyaluronic acid (HA) or ozone gas; 102 patients with early and moderate OA knee who presented with a ≥ 1-year history of knee pain and VAS score ≥4 were randomly separated into three groups. Group 1 (PRP group) received intra-articular injection of PRP × 2 doses, Group 2 (HA group) received a single dose of HA, and Group 3 (Ozone group) received ozone × four doses. Weight-bear- ing anteroposterior–lateral and Merchant's radiographs of both knees were evaluated. WOMAC and VAS scores were applied to all patients on first presentation and at 1, 3, 6 and 12 months.

At the end of the 1st month after injection, significant improvements were seen in all groups. In the 3rd month, the improvements in WOMAC and VAS scores were similar in Groups 1 and 2, while those in Group 3. At the end of the 1st month after injection, significant improvements were seen in all groups. In the 3rd month, the improvements in WOMAC and VAS scores were similar in Groups 1 and 2, while those in Group 3. The study concluded that in the management of mild–moderate knee OA, PRP was more successful than HA and ozone injections, as the application alone was sufficient to provide at least 12 months of pain-free daily living activities. [4]

In the 2017 study conducted by Carlos Ce´sar Lopes de Jesus et al, the aim of the study was to determine the effectiveness of oxygen-ozone injections on knee osteoarthritis concerning pain reduction, joint functional improvement, and quality of life. The efficacy outcomes for knee OA were the Visual Analogue Scale (VAS), Lequesne Index, Timed Up and Go Test (TUG Test), SF-36, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Geriatric Pain Measure (GPM). After 8 weeks of treatment, ozone was more effective than the placebo. [5]

In the 2018 study conducted by Arash Babaei-Ghazani & Saeedeh Najarzadeh & Korosh Mansoori & Bijan Forogh & Seyed Pezhman Madani & Safoora Ebadi & Hamid Reza Fadavi & Bina Eftekharsadat, the purpose was to compare the effects of ultrasound-guided corticosteroid injection with O2–Ozone injection in patients with knee OA. The study was a double-blind randomised clinical trial was performed on 62 patients. Outcome measures included the Western Ontario and McMaster Universities Osteoarthritis (WOMAC), knee flexion range of motion (ROM), effusion in ultrasound images of the supra patellar recess, and visual analog scale (VAS), which were evaluated before injection, 1 week, 1 month, and 3 months after the treatment. The significant improvements seen in VAS and WOMAC scores 3 months after treatment were in favour of the oxygen–ozone group when compared to the steroid group ($P = 0.041$ vs $P = 0.19$). There was no significant difference between the two groups in ROM and joint effusion seen under ultrasound. In the oxygen–ozone-receiving group, joint effusion was decreased significantly ($p < 0.001$). Both steroid and oxygen– ozone injections are effective in patients with knee osteoarthritis. The study concluded that the effects of oxygen–ozone injection lasts longer than those of steroid injection to the knee joint. [6]

In the 2021 study conducted by Raeissadat et al, comparing the effects of intra-articular injection of Platelet Rich Plasma (PRP), Plasma Rich in Growth Factor (PRGF), Hyaluronic Acid (HA), and Ozone in knee osteoarthritis, 238 patients with mild to moderate knee OA were randomised into 4 groups of IAIs: HA (3 doses weekly), PRP (2 doses with 3 weeks interval), PRGF (2 doses with 3 weeks interval), and Ozone (3 doses weekly). Our outcome measures were the mean changes from baseline (immediately from the first injections) until 2,6, and 12 months post intervention in scores of visual analog scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Lequesne index. In the 200 patients finally assessed, at 2 months follow up, significant improvement of pain, stiffness, and function were seen in all groups compared to the baseline, but the ozone group had the best results ($P < 0.05$). In 6 month follow up HA, PRP, and PRGF groups demonstrated better therapeutic effects in all scores in comparison with ozone ($P < 0.05$). At the end of the 12th month, only PRGF and PRP groups had better results versus HA and ozone groups in all scores ($P < 0.05$). Despite the fact that ozone showed better early results, its effects begin to wear off earlier than other products and ultimately disappear in 12 months. [7]

The 2018 study by Gaballa et al, the efficiency of intra-articular injection of PRP and ozone therapy in the treatment of patients with primary KOA were compared to a basic rehabilitation program.60 patients with KOA were randomly classified into 3 groups (20 patients each); group I received intra-articular PRP injection, group II received ozone and group III performed a rehabilitation program.All patients were assessed by visual analogue scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and 6-min walk test at baseline and after 1 and 3 months.The study concluded that use of autologous PRP is an efficient treatment of KOA and resulted in better outcomes than ozone therapy up to 3 months. [8]

MATERIALS AND METHODOLOGY

Study Design

The present study is a prospective cohort study conducted at the Department of Orthopaedics, Santosh Medical College & Hospital, Ghaziabad (UP).

Study Period:

The study will be conducted from February 2021 to April 2022.

Study Setting:

The study will be carried out among patients in the age bracket of 45 to 85 years) presenting with primary Osteoarthritis of one or both knees and meeting the inclusion criteria.

Study Area:

Department of Orthopaedics, Santosh Medical College & Hospital, Ghaziabad (UP)

SELECTION OF CASES

Inclusion Criteria:

1. Patients reporting to Santosh Medical College, Department of Orthopaedics.
2. Male and Female patient between the age of 45 and 85 years of age
3. Patient presenting with Kellegren- Lawrence Grade 1 , 2, and early 3 Osteoarthritis of the Knee Joint; unilateral and/ or bilateral knees.
4. Patient agreeing for follow-up at the decided intervals
5. Patients willing to participate in the detailed questionnaire process for the study.
6. Patients agreeing with "signed and informed consent"

Exclusion Criteria:

1. All patients below 45 years and above 85 years.
2. History of any previous intra articular knee fractures in the affected knee.
3. History of surgical intervention in the affected knee.
4. Pregnant patients, or immune-compromised patients
5. Patients with any coagulopathy and/or thrombocytopenia.
6. Patients with a history of any Cardiovascular instability or any other severe disease.
7. In the case of Ozone treatment: deficit of glucose-6- phosphate dehydrogenase, pregnancy, hyperthyroidism, treatment on angiotensin converting enzyme inhibitors, or allergy to Ozone.
8. Patients with uncontrolled Diabetes Mellitus.
9. Patient with neurological condition
10. Lignocaine Hypersensitivity
11. Patients with inflammatory knee conditions
12. Patients refusing to give informed consent

Ethical Approval:

Ethical Approval will be obtained from the Institutional Ethical Committee after explaining the Aim and Objectives of the Study.

Informed Consent:

A written Informed Consent will be obtained from each patient before clinical examination as well as data collection. The involvement of the subject will be voluntary and deliberate. All aspects of the study and requisite patient involvement shall be explained to the patients in the language that they understand. *(Consent form attached)*

SAMPLE SIZE

At 95% confidence level and taking mean % improvement in VAS score after Ozone+PRP treatment in SSPE subjects as 81.13% with standard deviation of 17.16 (Yeprem L et al) with allowable error of 5, **the sample size estimated was 46 using the formula :**

$$n = Z^2_{1-\alpha/2}\ \sigma^2/E^2$$

Where n = sample size

Zα = 1.96 value of the standard normal variate corresponding to level of significance alpha 5%

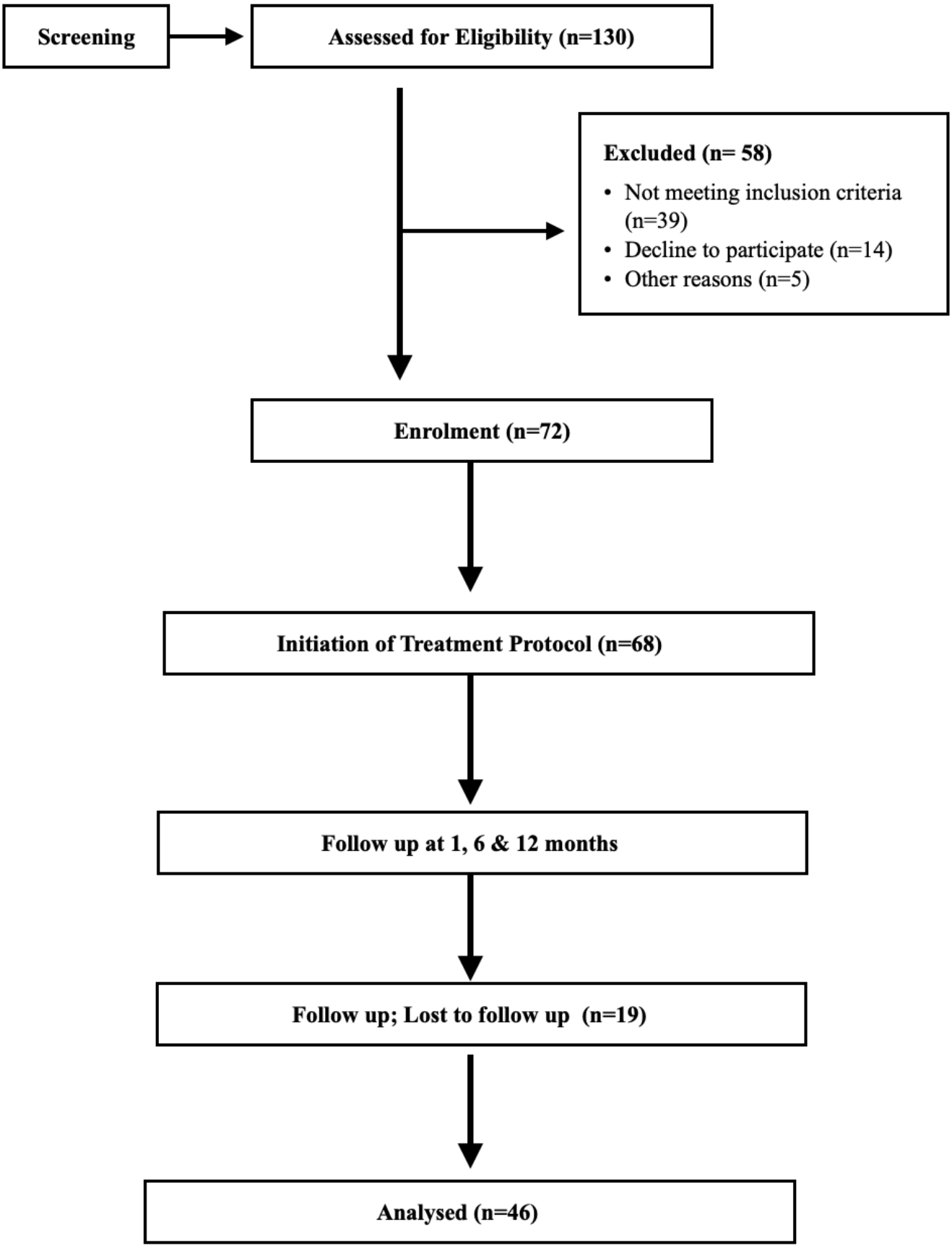
σ - Standard deviation

E- allowable error

Statistical Analysis

The collected data will be entered in Microsoft Excel and then will be analysed and statistically evaluated using SPSS-21 version.

Normality of each variable will be assessed by using the Kolmogorov- Simirnov test and Shapiro-Wilk test. Quantitative data will be expressed by mean, standard deviation or median with interquartile range and depends on normal distribution, difference between two means will be tested by student t test or Mann Whitney U test while for pre-post comparison paired t test or Wilcoxon signed rank test will be used. Qualitative data will be expressed in percentage and difference between the proportions will be tested by chi square test or Fisher's exact test. 'P' value less than 0.05 would be considered statistically significant.


Screening
Assessed for Eligibility (n=130)
Excluded (n= 58)
• Not meeting inclusion criteria (n=39)
• Decline to participate (n=14)
• Other reasons (n=5)
Enrolment (n=72)
Initiation of Treatment Protocol (n=68)
Follow up at 1, 6 & 12 months
Follow up; Lost to follow up (n=19)
Analysed (n=46)

Flowchart of the study: Screening, assessment, treatment and follow-up of patients with OA. *n* = number of patients.

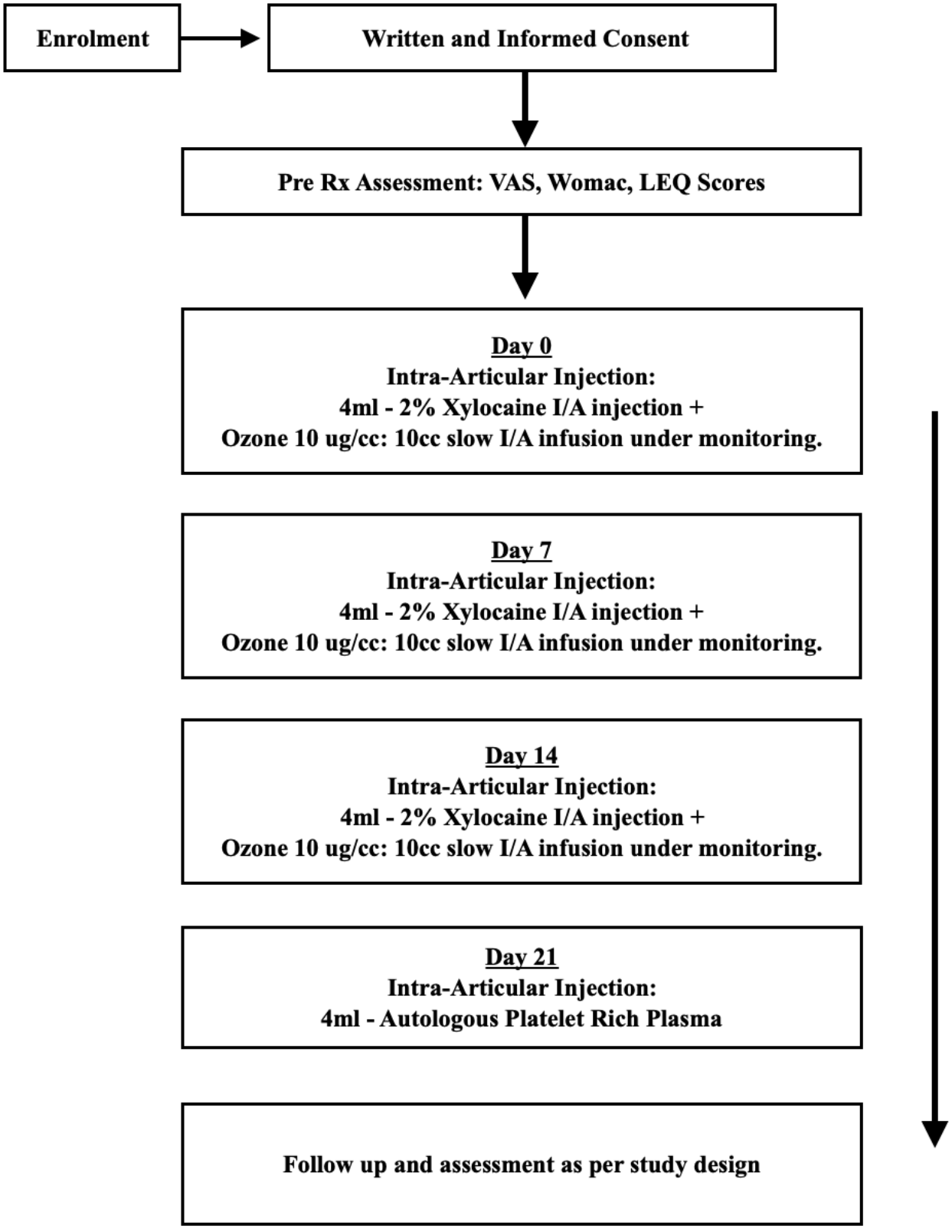

Enrolment
Written and Informed Consent
Pre Rx Assessment: VAS, Womac, LEQ Scores
Day 0
Intra-Articular Injection:
4ml - 2% Xylocaine I/A injection +
Ozone 10 ug/cc: 10cc slow I/A infusion under monitoring.
Day 7
Intra-Articular Injection:
4ml - 2% Xylocaine I/A injection +
Ozone 10 ug/cc: 10cc slow I/A infusion under monitoring.
Day 14
Intra-Articular Injection:
4ml - 2% Xylocaine I/A injection +
Ozone 10 ug/cc: 10cc slow I/A infusion under monitoring.
Day 21
Intra-Articular Injection:
4ml - Autologous Platelet Rich Plasma
Follow up and assessment as per study design

Figure : Ozone + Platelet Rich Plasma Therapy Treatment protocol

Intra Articular Ozone Infusion

Method : Slow intra articular infusion

Dosage : 10cc of Medical Ozone at a concentration of 10 ug/cc.

Route of administration : Intra-articular infusion using the anterio medial access point and anterio lateral access point to the knee joint. In few cases where these entry points could not be clearly ascertained, other appropriate approaches was used to access the knee joint.

Patient Position : Sitting position; with knee hanging freely.

Ozone Preparation : Using standard medical ozone generation equipment. Obtained and handled under aseptic and sterile conditions.

Administration Protocol :

First Dose - Day 0 : I/A Injection
4ml - 2% Xylocaine I/A injection + Ozone 10 ug/cc: 10cc slow I/A infusion under monitoring.

Second Dose - Day 7 : I/A Injection
4ml - 2% Xylocaine I/A injection + Ozone 10 ug/cc: 10cc slow I/A infusion under monitoring.

Third Dose - Day 14 : I/A Injection
4ml - 2% Xylocaine I/A injection + Ozone 10 ug/cc: 10cc slow I/A infusion under monitoring.

Patient kept under observation and monitoring for 30 minutes post injection for any adverse effects.

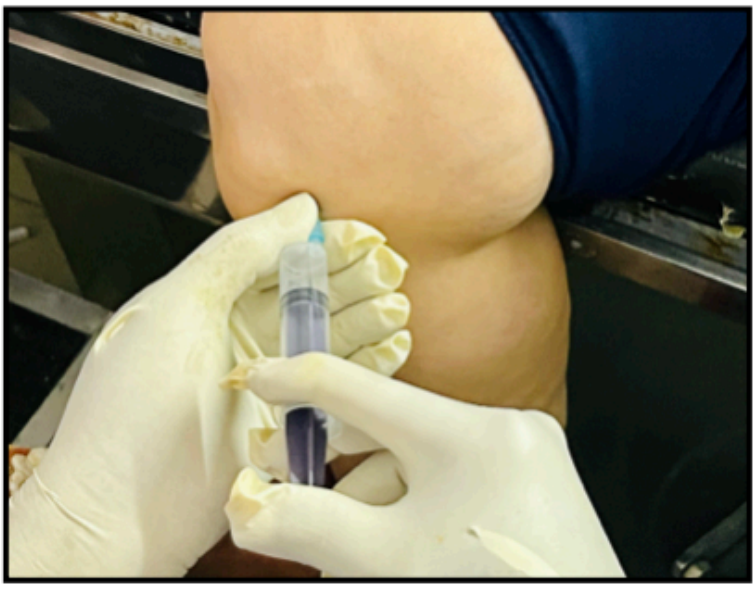


Figure : Intra-articular injection of Ozone

Figure : Apparatus for medical ozone generation

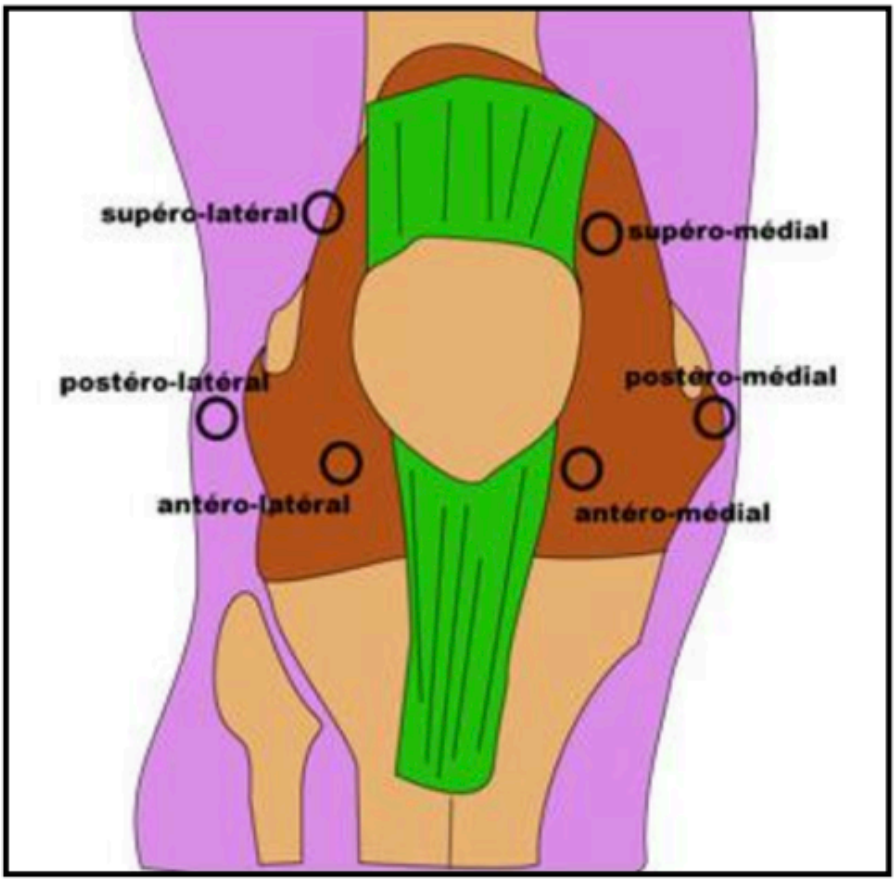


Figure : Access points to the knee joint.

Intra Articular Platelet Rich Plasma (PRP)
Preparation and Injection

Method : Intra articular injection

Dosage : 3-4 ml of freshly prepared Autologous Platelet Rich Plasma

Route of administration : Intra-articular infusion using the anterio medial access point and anterio lateral access point to the knee joint. In few cases where these entry points could not be clearly ascertained, other appropriate approaches was used to access the knee joint.

Patient Position : Sitting position; with knee hanging freely.

PRP Preparation : Using patient's own blood sample for autologous PRP generation using specialised PRP kits and centrifuge equipment. Activated with Calcium Gluconate.

Administration Protocol :

Fourth Dose - Day 21 : I/A Injection
3 - 4ml - Autologous Platelet Rich Plasma.

Specialised equipment : Cooled centrifuge mechanism with specialised tubes for PRP preparation. Intra-procedural cooling prevents heat lysis of cellular components during the centrifuge process. PRP collection tubes used in this study were US FDA approved.

Centrifuge Parameter Limits for PRP preparation:

Max. Speed (rpm)	6000
Max. RCF (g)	5070
Max. Tube Size (ml)	100
Max. Capacity (ml)	400
Min. Temperature (°C)	-8
#Noise	≤ 60 dB

Figure : Cooled PRP preparation centrifuge machines

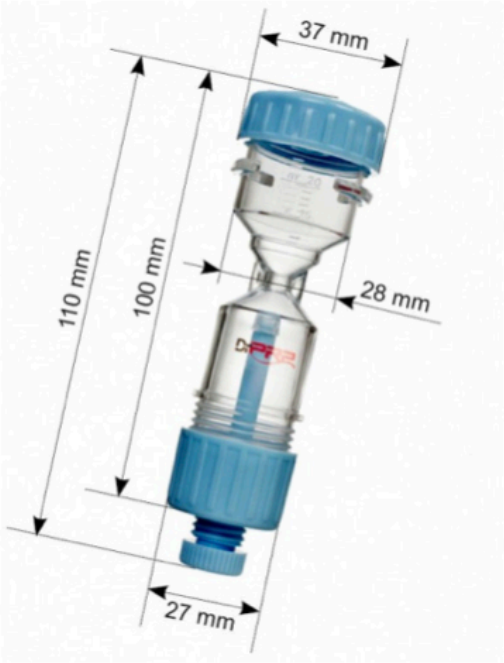


Figure : Specialised Tube

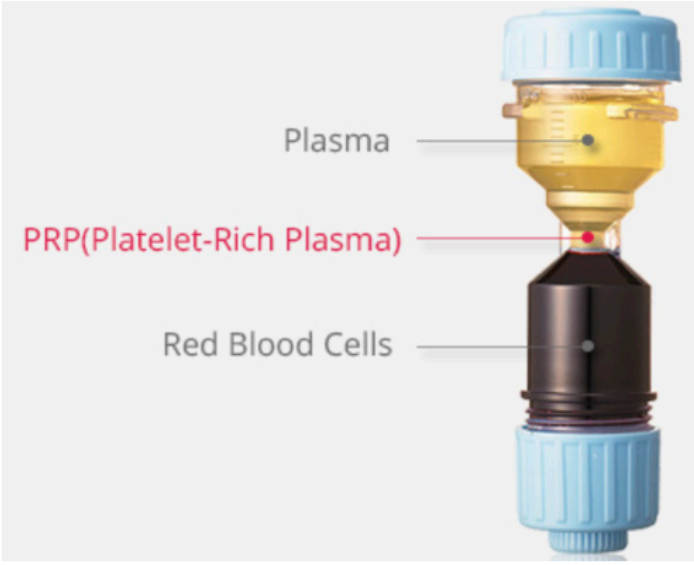


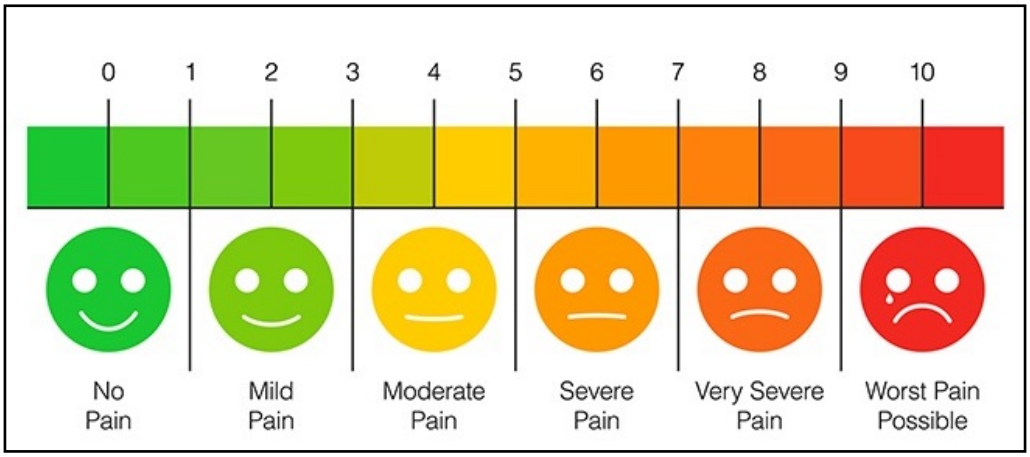
Figure : Post centrifuge component separation.

Figure : Autologous PRP obtained

Figure : I/A knee joint PRP Injection

Methods of Outcome Assessment :

1. The Visual Analogue Scale (VAS) is a psychometric response scale that can be used in questionnaires. It is a measurement instrument for subjective characteristics or attitudes that cannot be directly measured. When responding to a VAS item, respondents specify their level of agreement to a statement by indicating a position along a continuous line between two end points.



VAS Scores obtained Pre Treatment and at 1 Month, 6 Months and 12 Months intervals.

Methods of Outcome Assessment :

2. WOMAC Score :

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is a widely used, proprietary set of standardised questionnaires used by health professionals to evaluate the condition of patients with osteoarthritis of the knee and hip, including pain, stiffness, and physical functioning of the joints.

It can be self-administered and was developed at Western Ontario and McMaster Universities in 1982.

Higher scores indicate worse pain, stiffness, and functional limitations.

The WOMAC measures five items for pain (score range 0–20), two for stiffness (score range 0–8), and 17 for functional limitation (score range 0–68). Physical functioning questions cover everyday activities such as stair use, standing up from a sitting or lying position, standing, bending, walking, getting in and out of a car, shopping, putting on or taking off socks, lying in bed, getting in or out of a bath, sitting, and heavy and light household duties.

A WOMAC test takes about 12 minutes.

The American College of Rheumatology notes that the test-retest reliability of the WOMAC varies for the pain, stiffness, and function sub-scales. The ACR says the pain sub-scale "has been variable across studies but generally meets the minimum standard." Reliability for the physical function scale "has been more consistent and stronger... but the stiffness sub-scale has shown low test-retest reliability."When used in clinical studies, the WOMAC pain and function sub-scales perform comparably or better than other tests in being responsive to change from experimental interventions, but this varies for the different sub-scales and types of intervention.

WOMAC Pain, Stiffness, Function and Total Scores obtained Pre Treatment and at 1 Month, 6 Months and 12 Months intervals.

Study WOMAC questionnaire attached

Methods of Outcome Assessment :

3. LEQ Knee Score : Index of Severity for Osteoarthritis of the Knee (Lequesne et al.)

Lequesne et al developed an index of severity for osteoarthritis for the knee (ISK). This can be used to assess the effectiveness of therapeutic interventions.

Sections for index: (1) Pain or discomfort (2) Maximum distance walked (3) Activities of daily living

Interpretation:

- Minimum points for each section: 0
- Maximum points for each section: 8
- Minimum index score: 0
- Maximum index score: 24

Index Score	Handicap
0	None
1-4	Mild
5-7	Moderate
8-10	Severe
11-13	Very Severe
>=14	Extremely Severe

References:

Lequesne M Mery C et al. Indexes of severity for osteoarthritis of the hip and knee. Scand J Rheumatology. 1987; Supplement 65: 85-89.

Lequesne M. Indices of severity and disease activity for osteoarthritis. Seminars in Arthritis and Rheumatism. 1991; 20 (supplement 2): 48-54.

Lequesne MG. The algofunctional indices for hip and knee osteoarthritis. J Rheumatol. 1997; 24: 779-781.

LEQ Knee Scores obtained Pre Treatment and at 1 Month, 6 Months and 12 Months intervals.

Study Lequesne [LEQScore] questionnaire attached

OBSERVATIONS & RESULTS

Table : Sex wise distribution of study subjects (n=46)

Sex	No.	%
Male	19	41.3
Female	27	58.7
Total	46	100

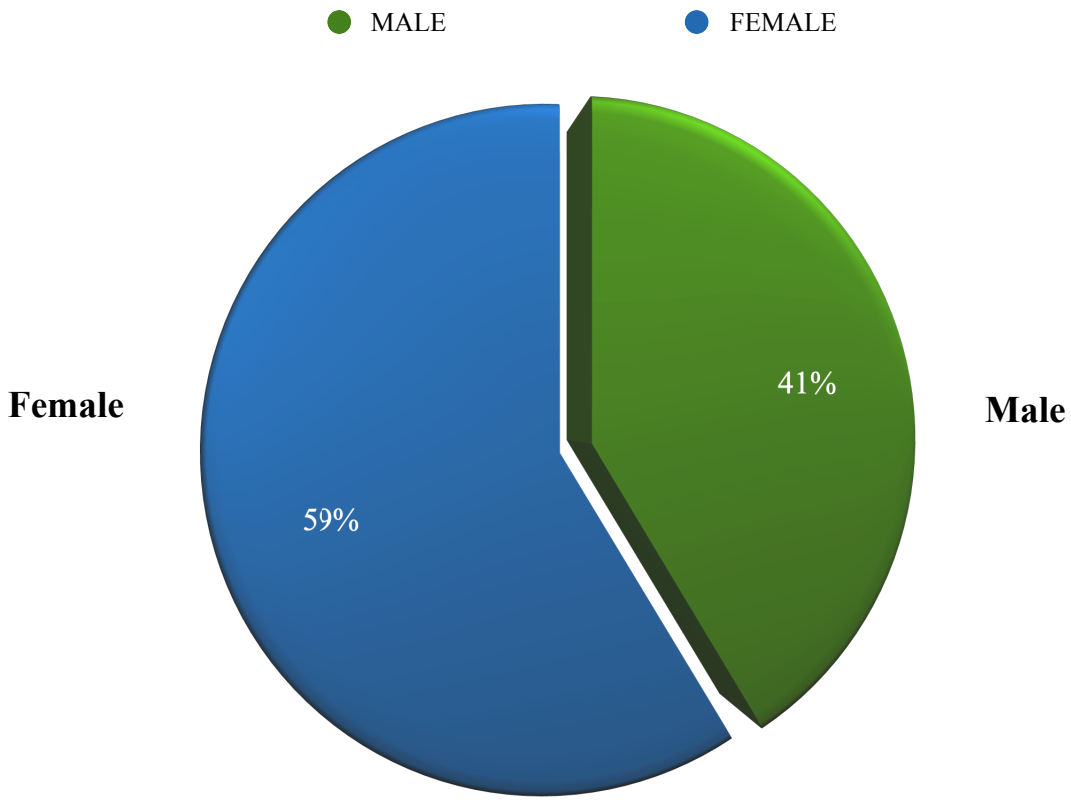


Figure : Sex wise distribution of study subjects (n=46)

Among the study population of a total of 46 patients, 19 patients [41.3%] were Male and 27 patients [58.7%] were Female.

Table : Age wise distribution of study subjects (n=46)

Age group	No.	%
45-54 years	11	23.9
55-64 years	15	32.6
65-74 years	12	26.1
75 years and above	8	17.4

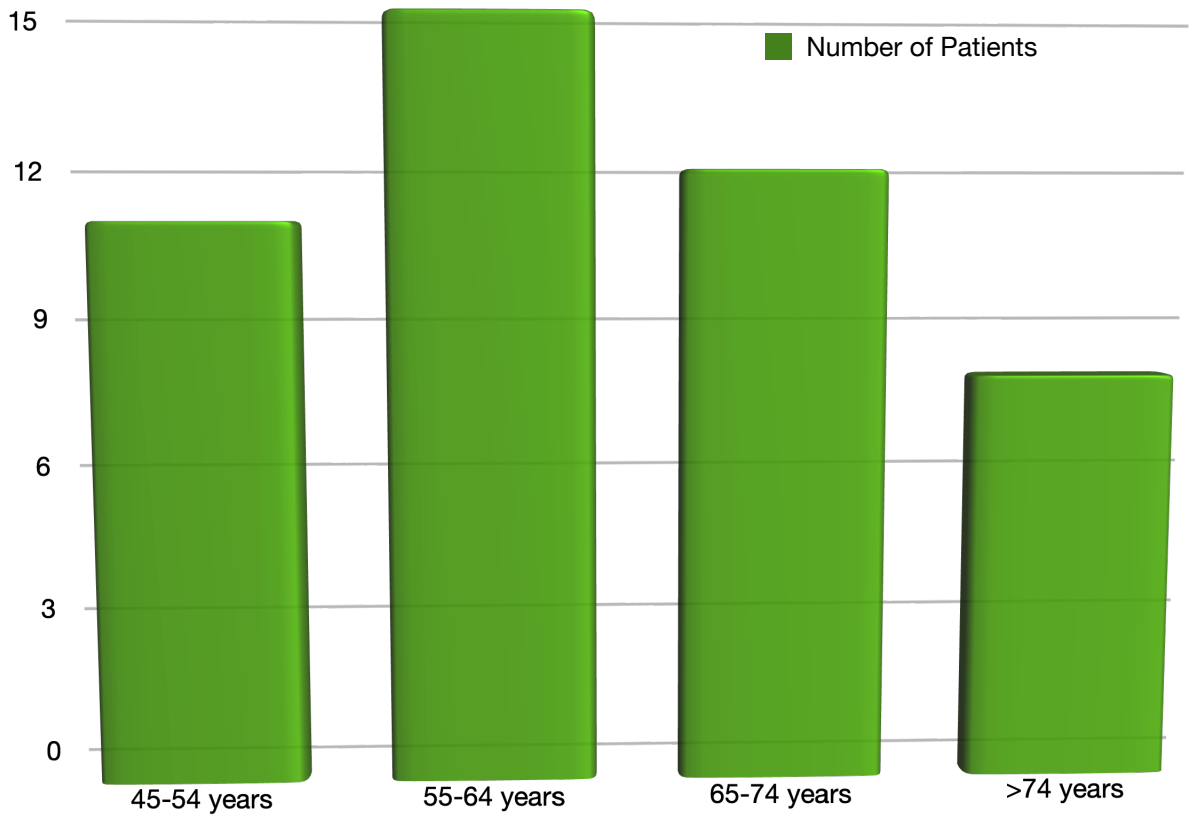


Figure : Age wise distribution of study subjects.

Among the patient study population, 11 patients (23.9 %)were between 45-54 years of age, 15 patients (32.6%) were between 55-64 years of age, 12 patients (26.1%) were between 65-74 years of age and 8 patients (17.4%) were above 74 years.

Table 3 : Side of Knee Involved (n=46)

Side of Knee	No.	%
B/L	19	41.3
Left	12	26.1
Right	15	32.6

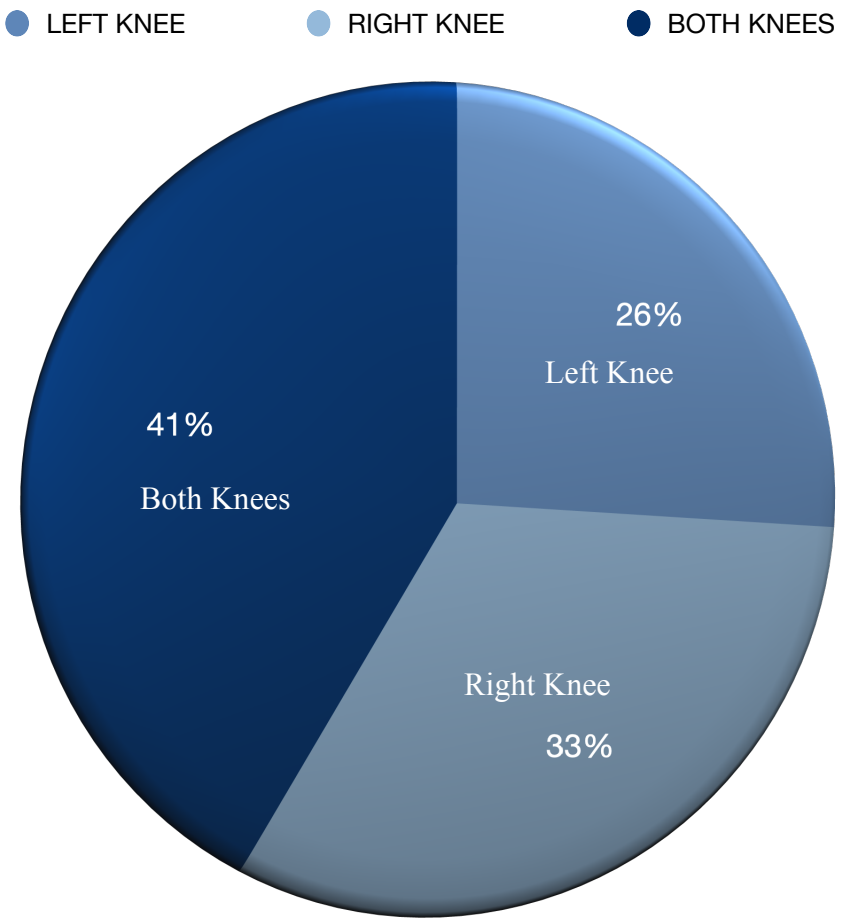


Figure : Side of Knee for intervention.

Among the patient study population, all presenting as per the inclusion criteria with K-L Grade 1/2 OA Knee, 19 patients (41.3 %) had intervention carried out in both knees, 12 patients (26.1 %) had Left knee only intervention, 15 patients (32.6%) had Right knee only intervention.

Table: Severity of Disease / Grade wise distribution of study subjects (n=46) based on the Kellegren- Lawrence Classification for OA Knee

Kellegren Lawrence OA grade	No.	%
1	8	17.4
2 & early 3	38	82.6

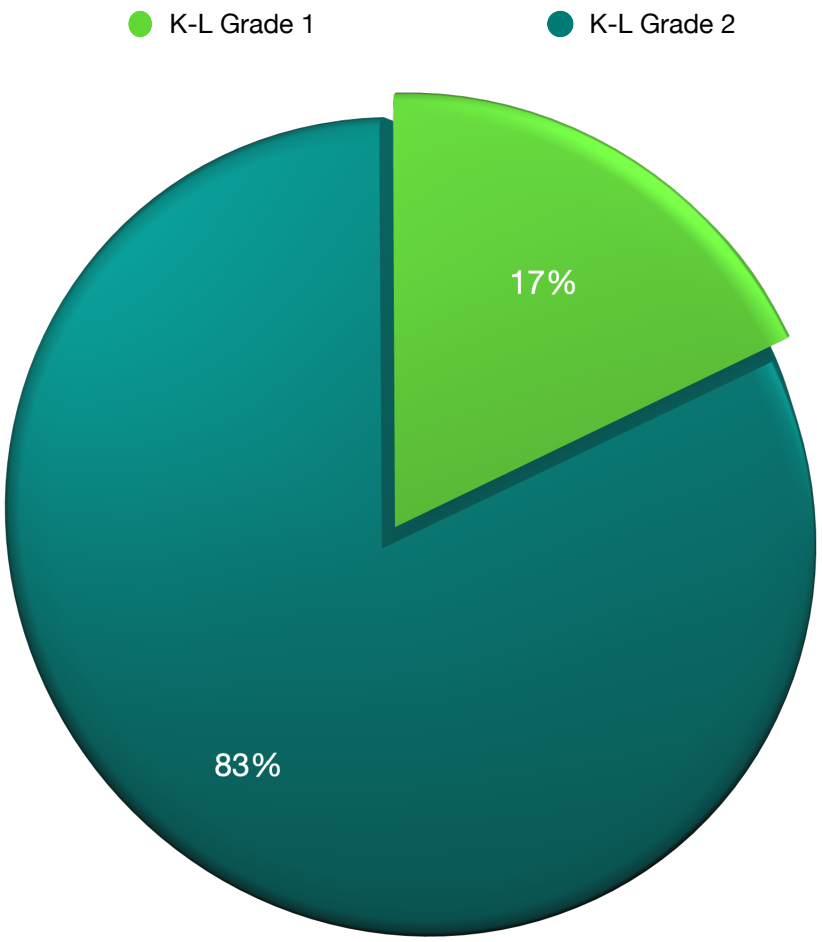


Figure : Table: Severity of Disease / Grade wise distribution of study subjects (n=46) based on the Kellegren- Lawrence Classification for OA Knee

Among the patient study population, 38 patients (82.6%) were Kellegren Lawrence Grade 2 OA Knee Cases and 8 patients (17.4%) were Kellegren-Lawrence Grade 1 OA Knee cases. Advanced grade presentations (K-L grad3 and above) were deemed unfit for isolated conservative management and were excluded from the study.

Table: Comparison of VAS score parameters from Pre-treatment to 1 month, 6 month and 12 month intervals (n=46)

VAS score	Mean Score	Standard Deviation	P value from Pre Rx
Pre	7.57	± 1.02	
T1	2.91	± 1.18	**<0.001**
T6	1.46	± 0.69	**<0.001**
T12	1.98	± 0.80	**<0.001**

The difference in the VAS Scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

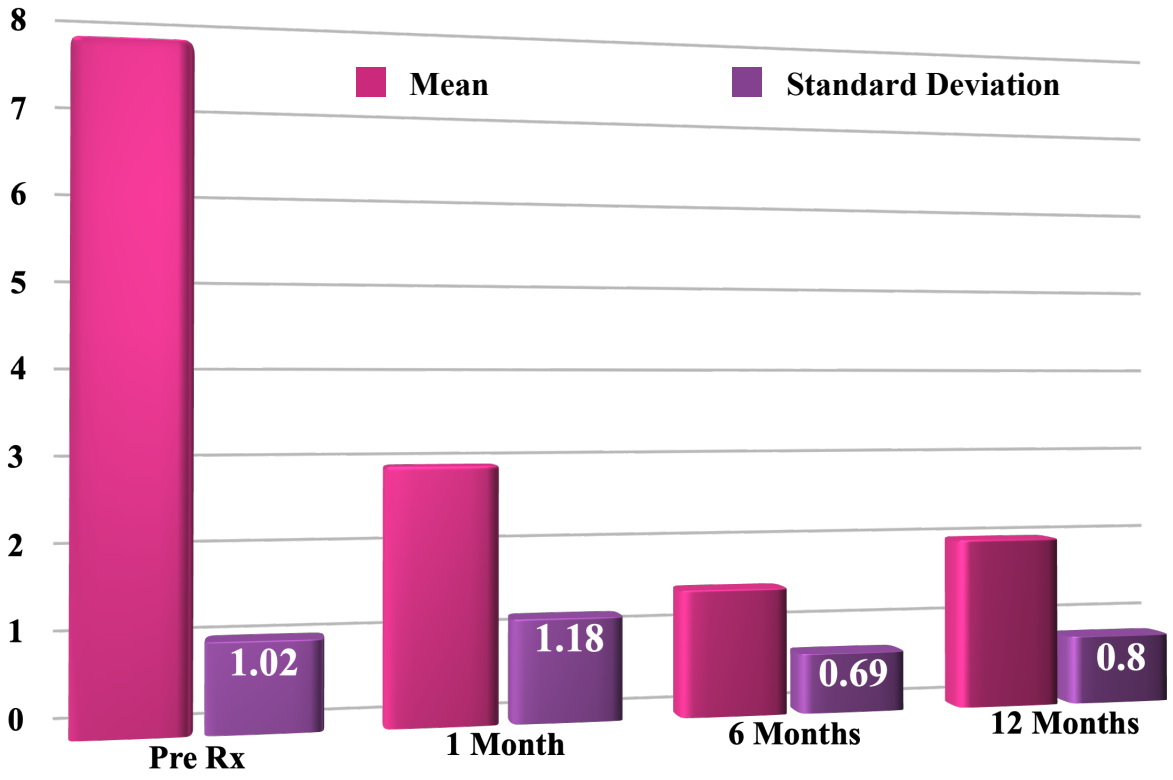


Figure : Comparison of VAS scores from Pre-treatment to 1 month, 6 month and 12 month intervals (n=46)

Visual Analog Scale; parameter for outcome progression and assessment; Pre Treatment , Mean VAS Score for the study population was 7.57 with a standard deviation of ± 1.02. VAS score at 1 month assessment were - Mean 2.91 with a standard deviation of ±1.18. VAS score at 6 month assessment were - Mean 1.46 with a standard deviation of ±0.69. VAS score at 12 months was - Mean 1.98 with a standard deviation of ±0.80.

Table: Comparison of VAS score parameters from Pre-treatment to 1 month, 6 month and 12 month intervals (n=46)

VAS score	Mean±SD	Median (IQR)	P value from Pre Rx
Pre	7.57±1.02	8 (7-8)	
T1	2.91±1.18	3 (2-3)	**<0.001**
T6	1.46±0.69	1 (1-2)	**<0.001**
T12	1.98±0.80	2 (1-2)	**<0.001**

The difference in the VAS Scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

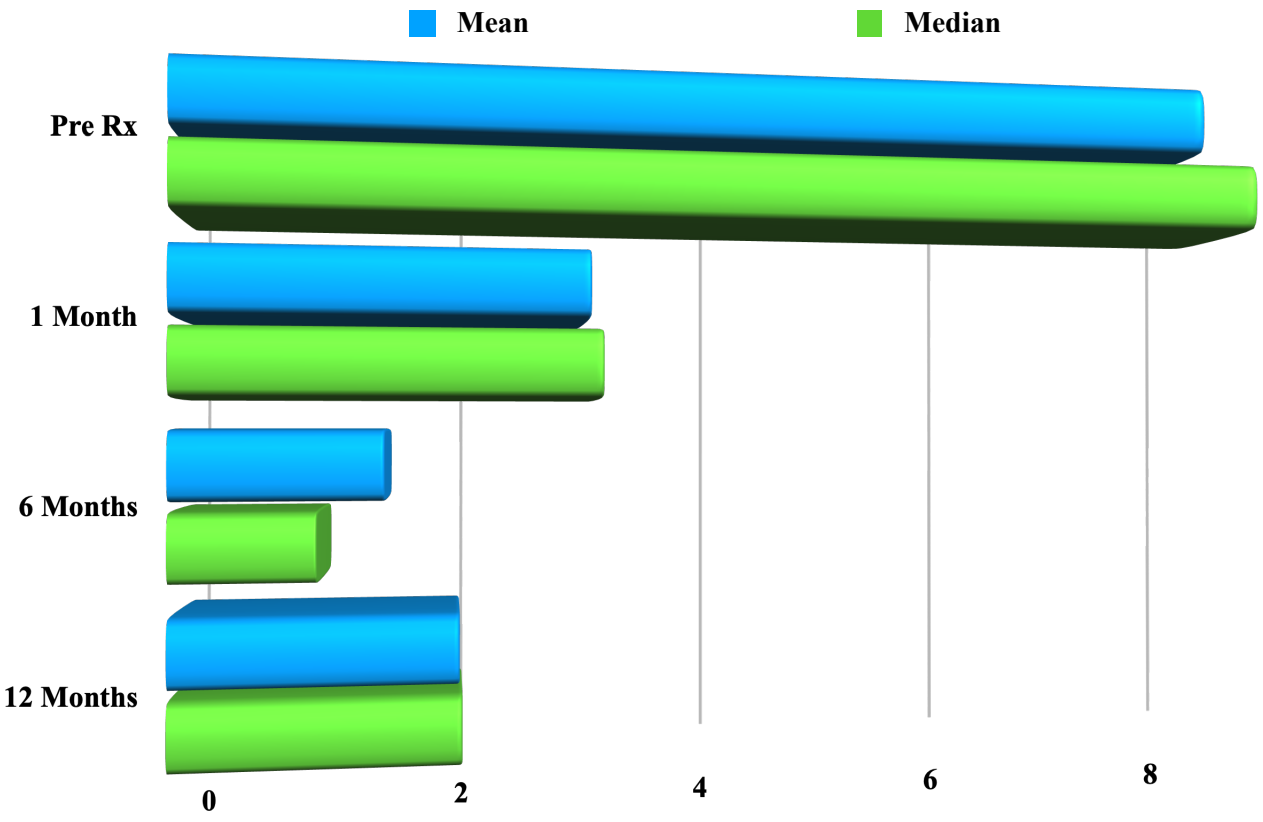


Figure : Comparison of VAS score Mean and Median value progression from Pre-treatment to 1 month, 6 month and 12 month intervals (n=46)

Visual Analog Scale; parameter for outcome progression and assessment; Pre Treatment , Mean VAS Score for the study population was 7.57±1.02 with a Median of 8. VAS score at 1 month assessment were - Mean 2.91±1.18 with a Median of 3. VAS score at 6 month assessment were - Mean 1.46±0.69 with a Median of 1. VAS score at 12 months was - Mean 1.98±0.80 with a Median of 2

Table : Comparison of WOMAC pain score from Pre treatment to 12 month (n=46)

WOMAC pain score	Mean Score	Standard Deviation	P value from pre
Pre	15.67	± 2.14	
T1	6.98	± 2.23	**<0.001**
T6	6.50	± 1.94	**<0.001**
T12	6.89	± 2.01	**<0.001**

The difference in the WOMAC Pain scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

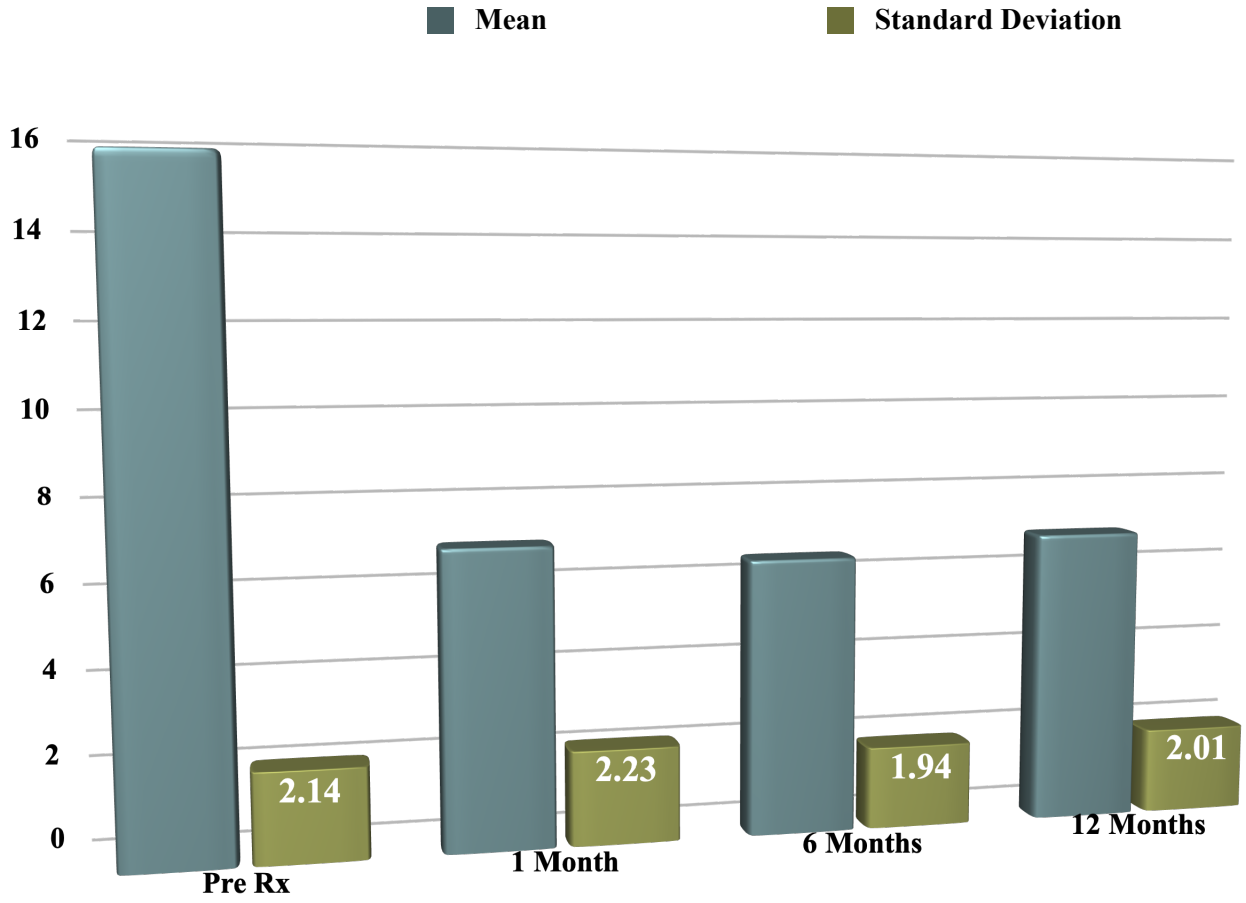


Figure : Comparison of Mean WOMAC pain score and the Standard Deviation from Pre treatment to 12 month (n=46)

The WOMAC Pain Score; parameter for outcome progression and assessment; Pre Treatment , Mean Womac pain score for study population was 15.67 with a standard deviation of ± 2.14. Womac pain score at 1 month assessment were - Mean 6.98 with a standard deviation of ± 2.23. Womac score at 6 month assessment were - Mean 6.50 with a standard deviation of ± 1.94. Womac score at 12 month assessment were - Mean 6.89 with a standard deviation of ± 2.01 Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC pain score from Pre treatment to 12 month (n=46)

WOMAC pain score	Mean±SD	Median (IQR)	P value from pre
Pre	15.67±2.14	16 (14-17)	
T1	6.98±2.23	7 (5—8.25)	**<0.001**
T6	6.50±1.94	6 (5-7.25)	**<0.001**
T12	6.89±2.01	7 (5-8)	**<0.001**

The difference in the WOMAC Pain scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

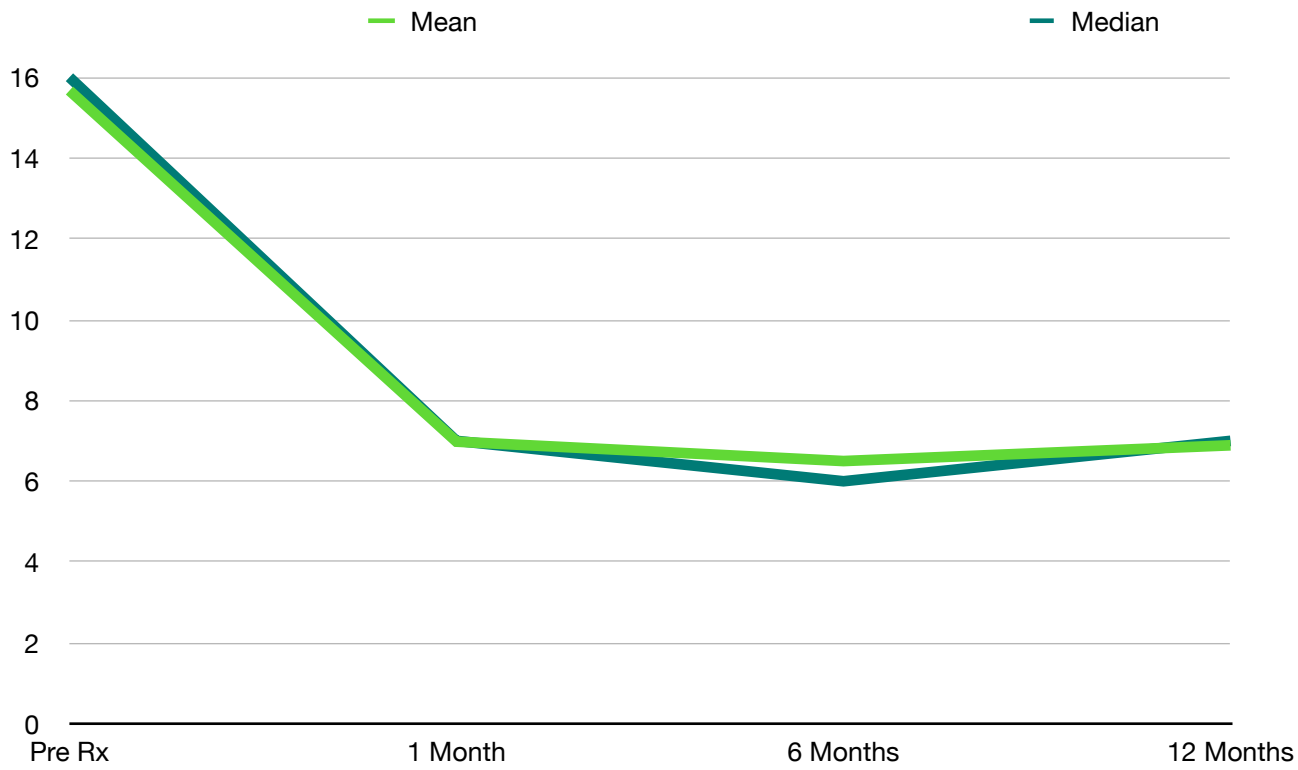


Figure : Comparison of Mean WOMAC pain score and the Median values from Pre treatment to 12 month (n=46)

The WOMAC Pain Score; parameter for outcome progression and assessment; Pre Treatment , Mean Womac pain score for study population was 15.67±2.14 with a Median of 16 (14-17). Womac pain score at 1 month assessment were - Mean 6.98±2.23 with a Median of 7 (5—8.25). Womac score at 6 month assessment were - Mean 6.50 with a Median of 6 (5-7.25). Womac score at 12 month assessment were - Mean 6.89 with a Median of 7 (5-8). Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC Stiffness score from Pre Treatment to 12 month (n=46)

WOMAC stiff score	Mean Score	Standard Deviation	P value from pre
Pre	5.80	± 1.84	
T1	3.0	± 1.28	**<0.001**
T6	2.85	± 1.11	**<0.001**
T12	3.11	± 1.01	**<0.001**

The difference in the WOMAC Stiffness scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

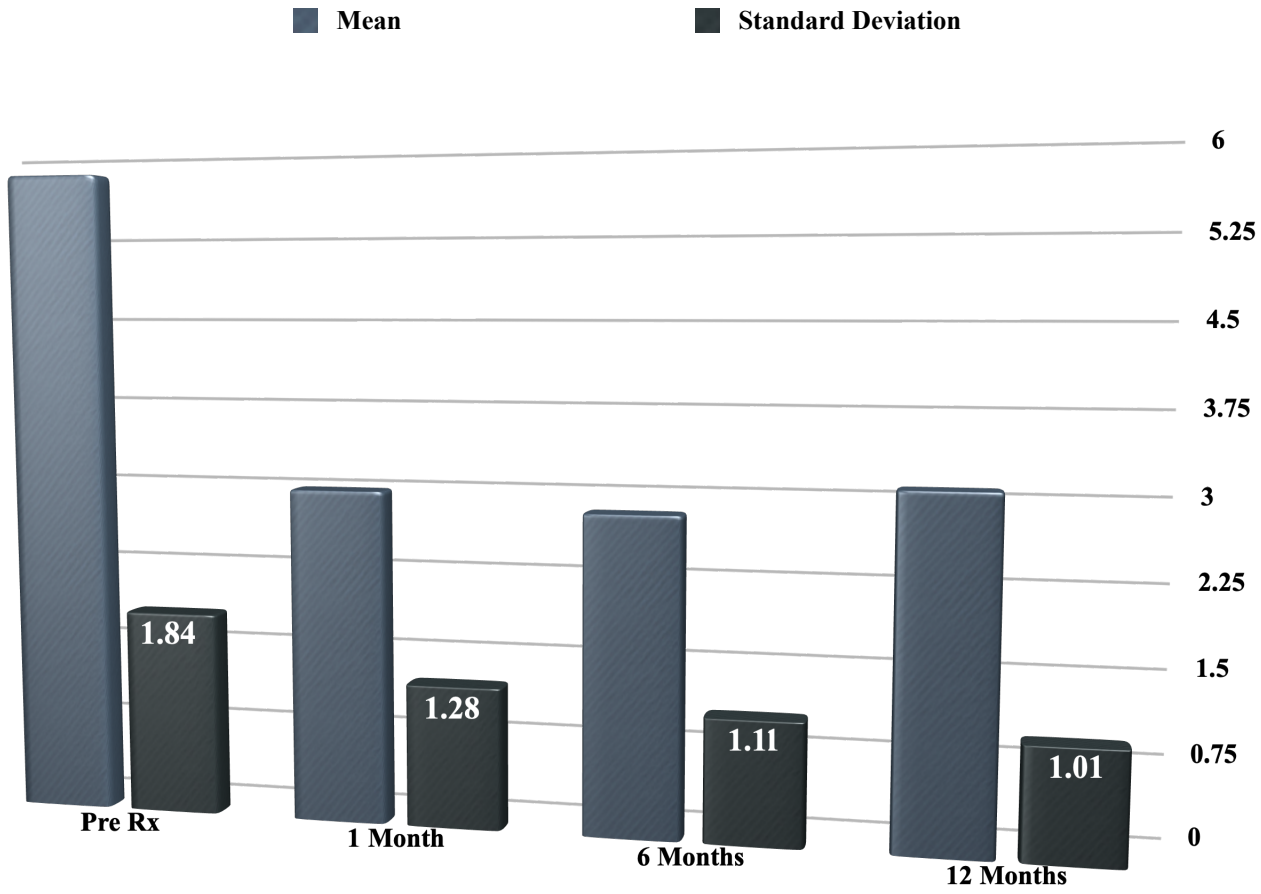


Figure : Comparison of Mean WOMAC Stiffness score and the Standard Deviation from Pre treatment to 12 month (n=46)

The WOMAC Stiffness Score; parameter for outcome progression and assessment; Pre Treatment, Mean Womac Stiffness score for study population was 5.80 with a standard deviation of ± 1.84. Womac Stiffness score at 1 month assessment were - Mean 3.0 with a standard deviation of ± 1.28. Womac Stiffness score at 6 month assessment were - Mean 2.85 with a standard deviation of ± 1.11. Womac Stiffness score at 12 month assessment were - Mean 3.11 with a standard deviation of ± 1.01 Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC Stiffness score from Pre Treatment to 12 month (n=46)

WOMAC stiff score	Mean±SD	Median (IQR)	P value from pre
Pre	5.80±1.84	6 (4.75-7)	
T1	3.0±1.28	3 (2-4)	**<0.001**
T6	2.85±1.11	3 (2-4)	**<0.001**
T12	3.11±1.01	3 (2-4)	**<0.001**

The difference in the WOMAC Stiffness scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

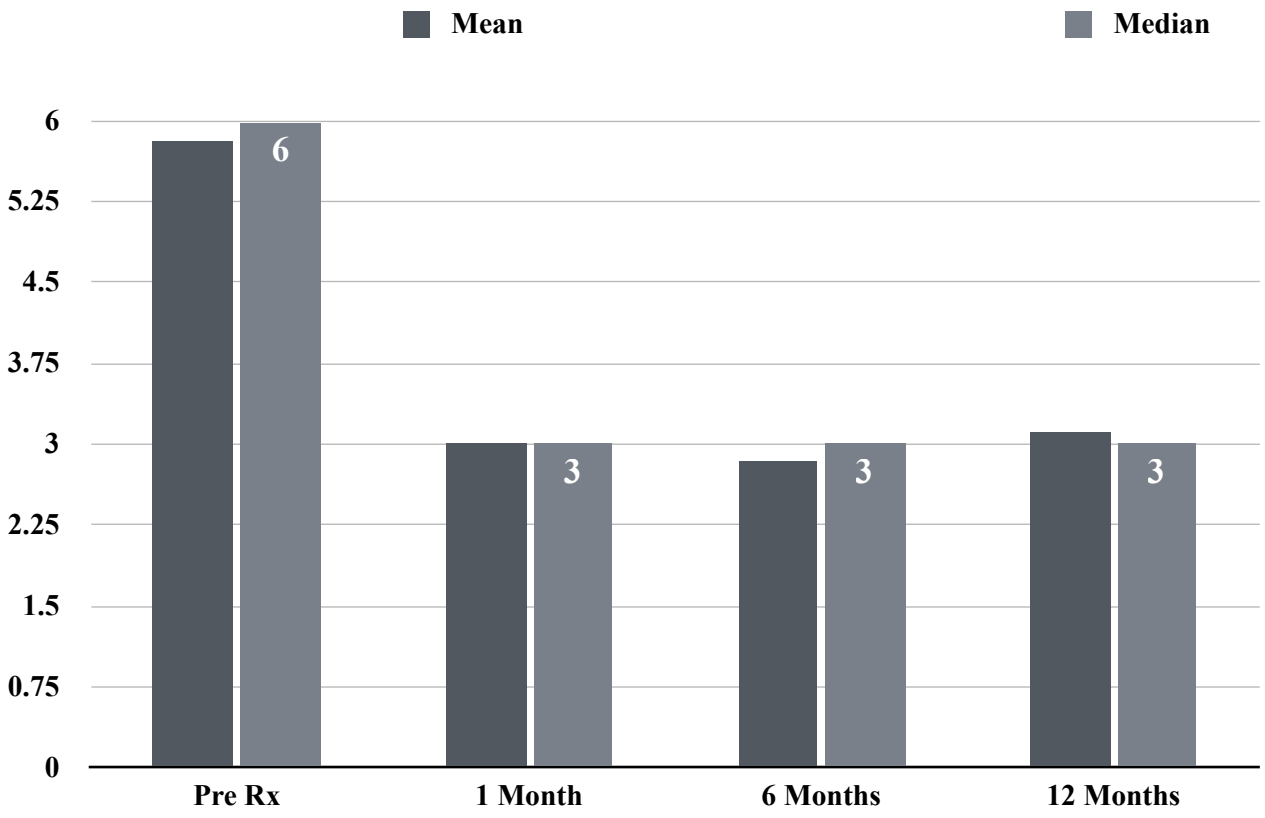


Figure : Comparison of Mean WOMAC Stiffness score and the Median values from Pre treatment to 12 month (n=46)

The WOMAC Stiffness Score; parameter for outcome progression and assessment; Pre Treatment, Mean Womac Stiffness score for study population was 5.80±1.84 with a Median of 6 (4.75-7). Womac Stiffness score at 1 month assessment were - Mean 3.0±1.28 with a Median of 3 (2-4). Womac Stiffness score at 6 month assessment were - Mean 2.85±1.11 with a Median of 3 (2-4). Womac Stiffness score at 12 month assessment were - Mean 3.11±1.01 with a Median of 3 (2-4). Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC Function score from Pre Treatment to 12 month (n=46)

WOMAC function score	Mean Score	Standard Deviation	P value from pre
Pre	53.22	± 8.24	
T1	19.43	± 4.59	**<0.001**
T6	18.98	± 4.15	**<0.001**
T12	20.67	± 3.94	**<0.001**

The difference in the WOMAC Function scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

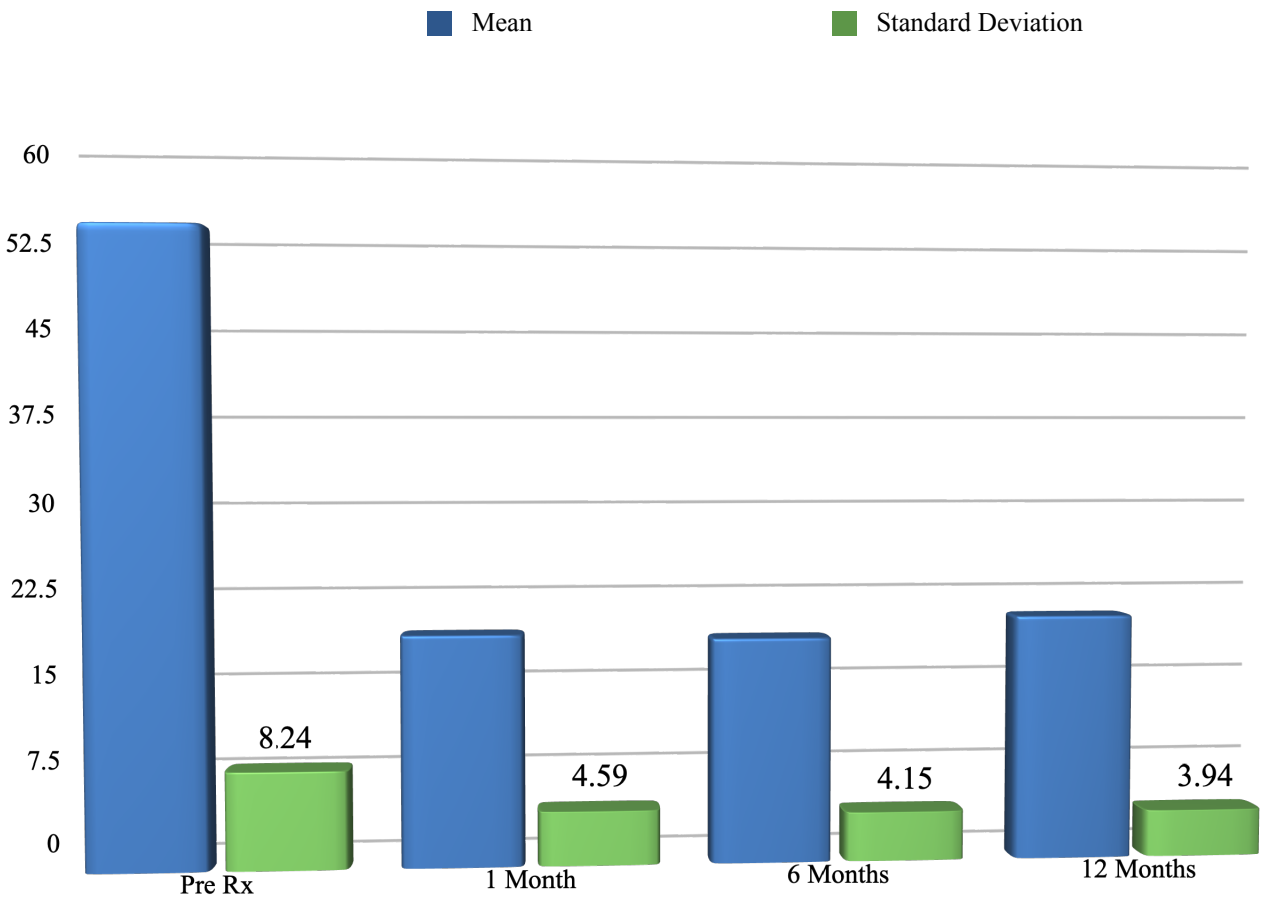


Figure : Comparison of Mean WOMAC Function score and the Standard Deviation from Pre treatment to 12 month (n=46)

The WOMAC Function Score; parameter for outcome progression and assessment; Pre Treatment, Mean Womac Function score for study population was 53.22 with a standard deviation of ± 8.24. Womac Function score at 1 month assessment were - Mean 19.43 with a standard deviation of ± 4.59. Womac Function score at 6 month assessment were - Mean 18.98 with a standard deviation of ± 4.15. Womac Function score at 12 month assessment were - Mean 20.67 with a standard deviation of ± 3.94 Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC Function score from Pre Treatment to 12 month (n=46)

WOMAC function score	Mean±SD	Median (IQR)	P value from pre
Pre	53.22±8.24	55 (48-59.25)	
T1	19.43±4.59	18 (16-22.25)	**<0.001**
T6	18.98±4.15	18 (15-22.5)	**<0.001**
T12	20.67±3.94	20 (16.75-25)	**<0.001**

The difference in the WOMAC Function scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

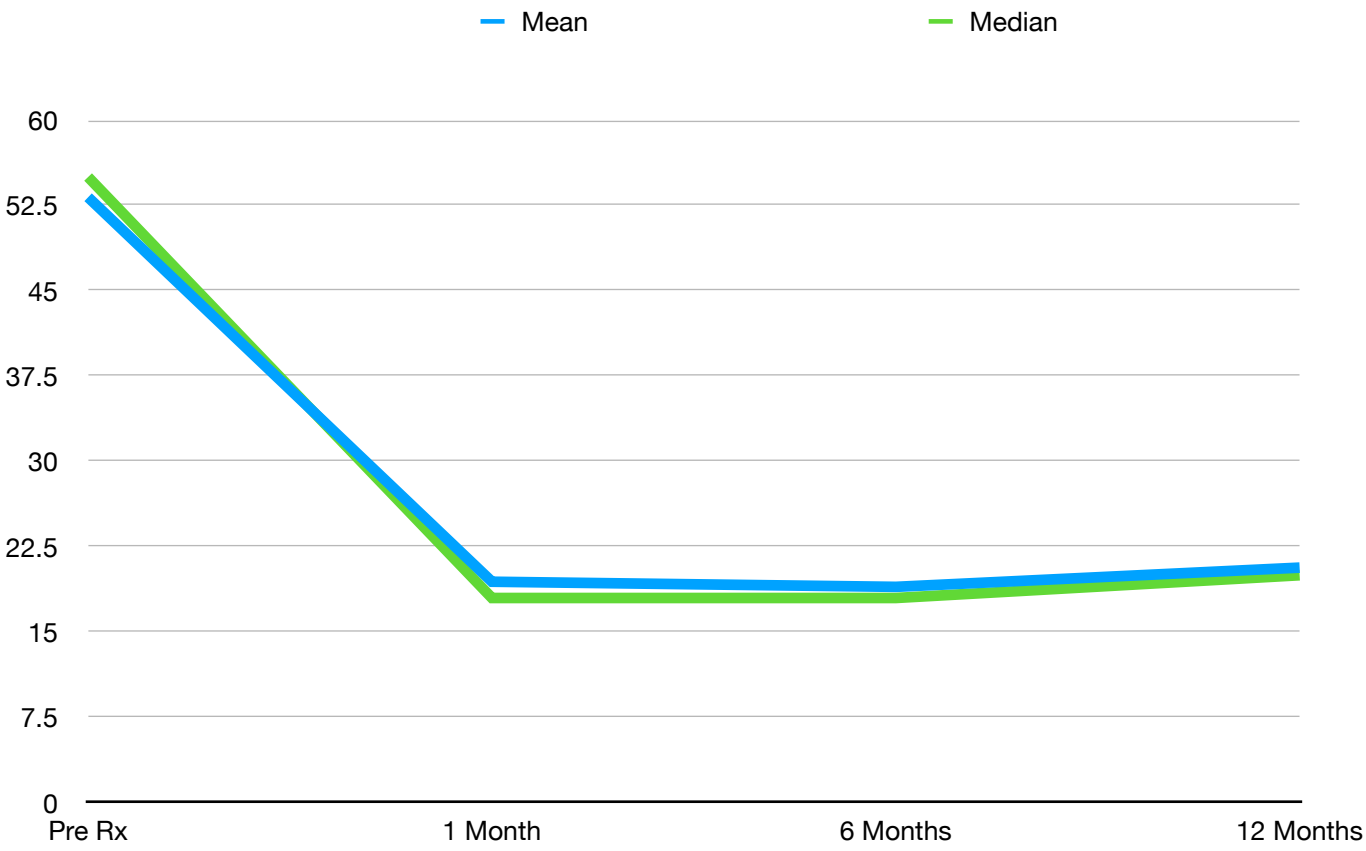


Figure : Comparison of Mean WOMAC Function score and the Median values from Pre treatment to 12 month (n=46)

The WOMAC Function Score; parameter for outcome progression and assessment; Pre Treatment, Mean Womac Function score for study population was 53.22±8.24 with a Median of 55 (48-59.25). Womac Function score at 1 month assessment were - Mean 19.43±4.59 with a Median of 18 (16-22.25). Womac Function score at 6 month assessment were - Mean 18.98±4.15 with a Median of 18 (15-22.5). Womac Function score at 12 month assessment were - Mean 20.67±3.94 with a Median of 20 (16.75-25). Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC Total score from pre treatment to 12 month (n=46)

WOMAC Total score	Mean Score	Standard Deviation	P value from pre
Pre	74.7	± 10.62	
T1	29.41	± 6.81	**<0.001**
T6	28.33	± 5.94	**<0.001**
T12	30.67	± 5.81	**<0.001**

The difference in the WOMAC Total scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

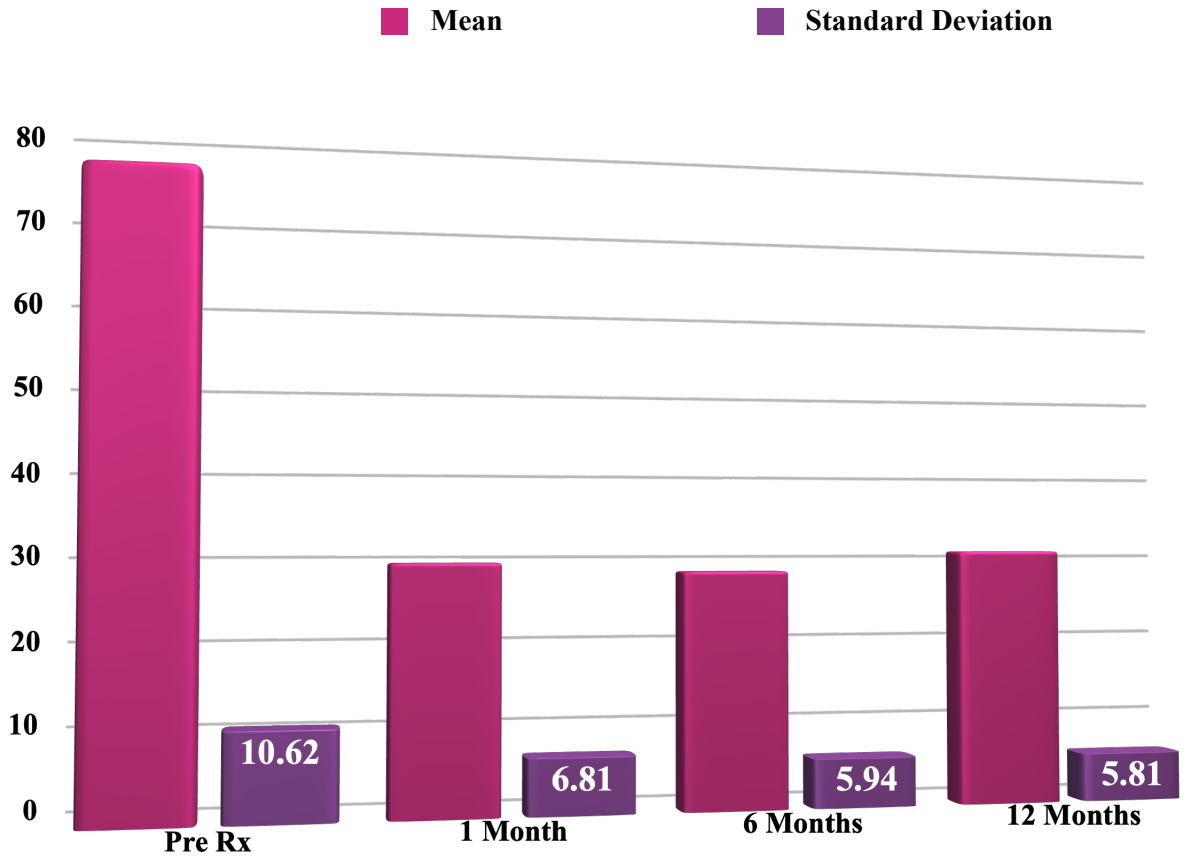


Figure : Comparison of Mean WOMAC Total Score and the Standard Deviation from Pre treatment to 12 month (n=46)

The WOMAC Score; parameter for outcome progression and assessment; Pre Treatment, Mean Womac score for study population was 74.7 with a standard deviation of ± 10.62. Womac score at 1 month assessment were - Mean 29.41 with a standard deviation of ± 6.81. Womac score at 6 month assessment were - Mean 28.33 with a standard deviation of ± 5.94 . Womac score at 12 month assessment were - Mean 30.67 with a standard deviation of ± 5.81 Differences from pre treatment value found to be statistically significant.

Table : Comparison of WOMAC Total score from pre treatment to 12 month (n=46)

WOMAC Total score	Mean±SD	Median (IQR)	P value from pre
Pre	74.7±10.62	77.5 (67.75-82)	
T1	29.41±6.81	28 (24.75-35.25)	**<0.001**
T6	28.33±5.94	27 (23-34.25)	**<0.001**
T12	30.67±5.81	30 (26-36)	**<0.001**

The difference in the WOMAC Total scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

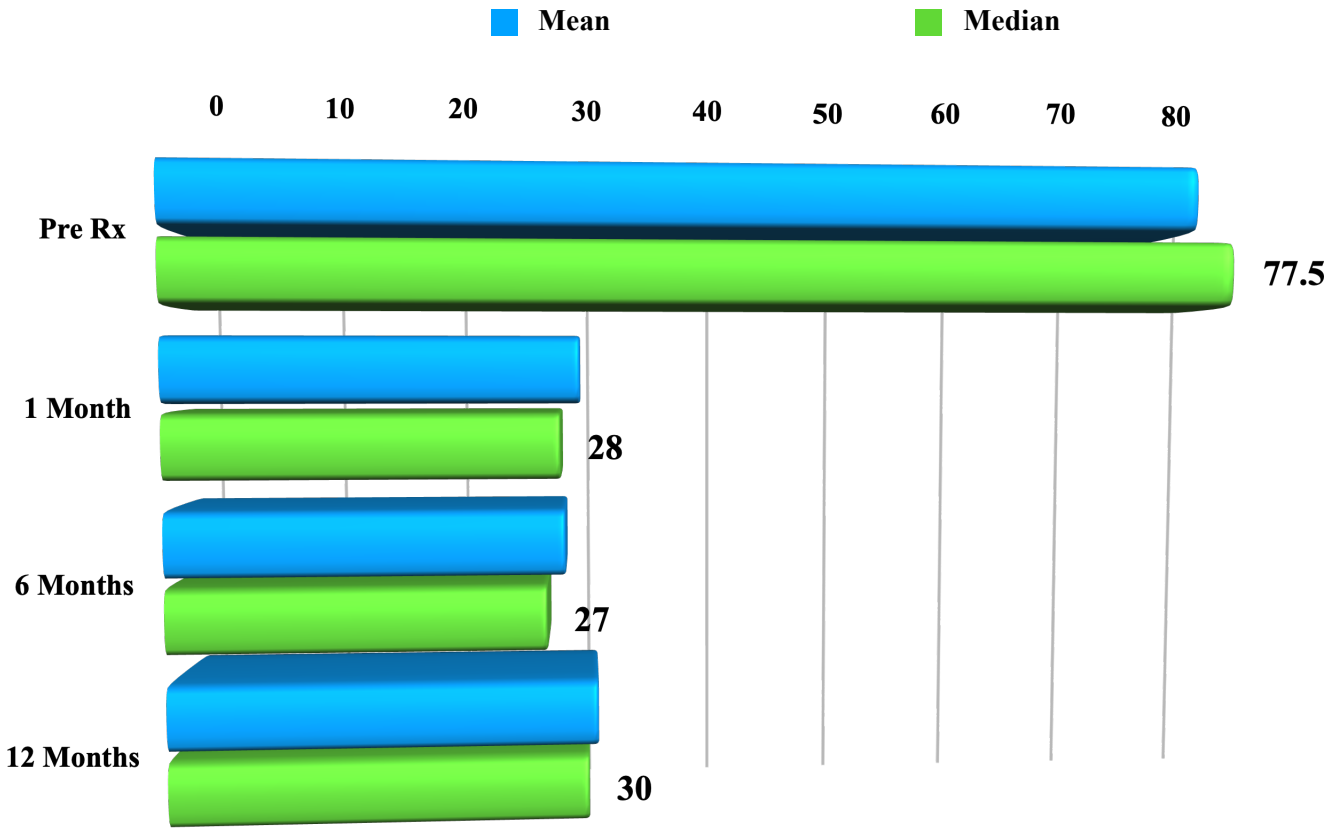


Figure : Comparison of Mean WOMAC Total score and the Median values from Pre treatment to 12 month (n=46)

The WOMAC Total Score; parameter for outcome progression and assessment; Pre Treatment, Mean Womac score for study population was 74.7±10.62 with a Median of 77.5 (67.75-82). Womac score at 1 month assessment were - Mean 29.41±6.81 with a Median of 28 (24.75-35.25). Womac score at 6 month assessment were - Mean 28.33±5.94 with a Median of 27 (23-34.25). Womac score at 12 month assessment were - Mean 30.67±5.81 with a Median of 30 (26-36). Differences from pre treatment value found to be statistically significant.

Table : Comparison of LEQ score from pre treatment to 12 month (n=46)

LEQ score	Mean Score	Standard Deviation	P value from pre
Pre	11.59	± 3.49	
T1	3.61	± 1.49	**<0.001**
T6	3.39	± 1.23	**<0.001**
T12	4.59	± 1.34	**<0.001**

The difference in the LEQ scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

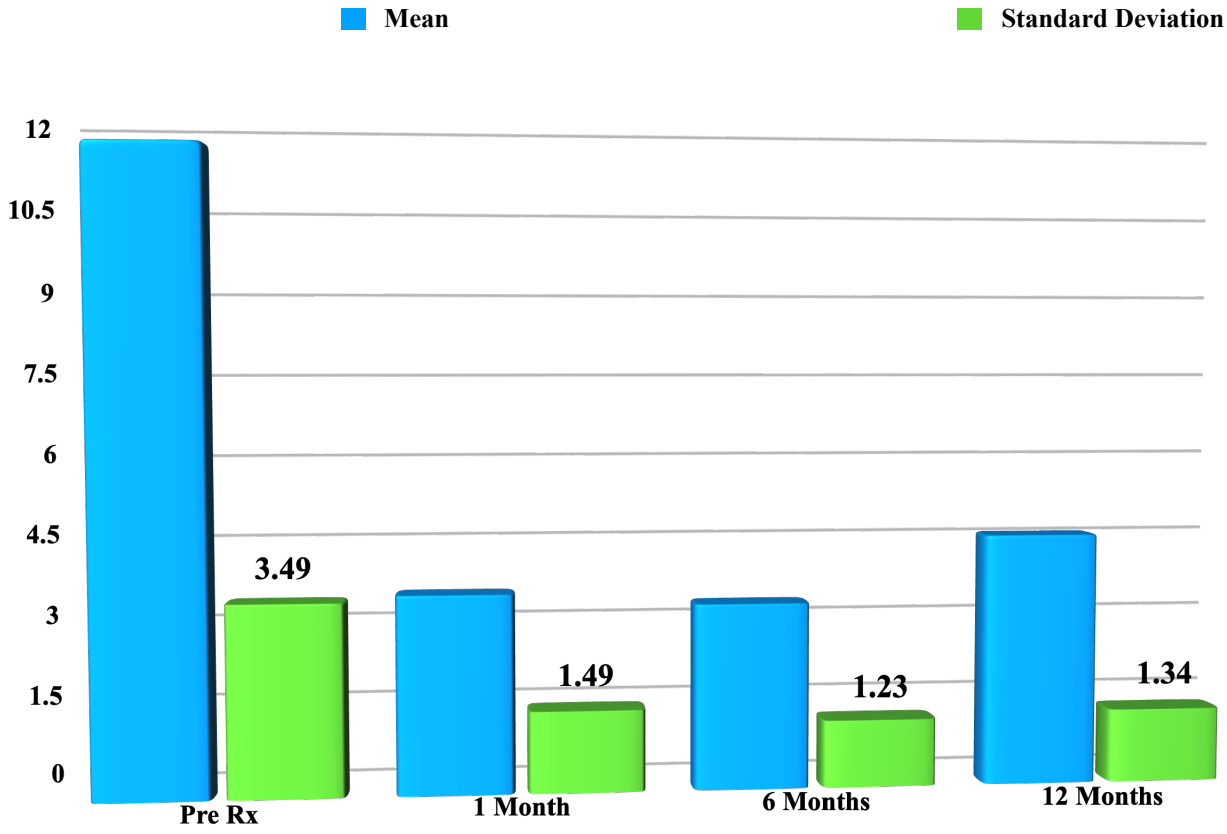


Figure : Table : Comparison of LEQ score from pre treatment to 12 month (n=46)

The LEQ Score; parameter for outcome progression and assessment; Pre Treatment, Mean LEQ score for study population was 11.59 with a standard deviation of ± 3.49. LEQ score at 1 month assessment were - Mean 3.61 with a standard deviation of ± 1.49. LEQ score at 6 month assessment were - Mean 3.39 with a standard deviation of ± 1.23 . LEQ score at 12 month assessment were - Mean 4.59 with a standard deviation of ± 1.34 Differences from pre treatment value found to be statistically significant.

Table : Comparison of LEQ score from pre treatment to 12 month (n=46)

LEQ score	Mean±SD	Median (IQR)	P value from pre
Pre	11.59±3.49	12 (8.75-14)	
T1	3.61±1.49	3.5 (2-5)	**<0.001**
T6	3.39±1.23	3.5 (2-4)	**<0.001**
T12	4.59±1.34	4.5 (4-6)	**<0.001**

The difference in the LEQ scores observed at 1,6 and 12 month intervals was statistically significant, [p value < 0.01] in comparison to Pre Treatment values.

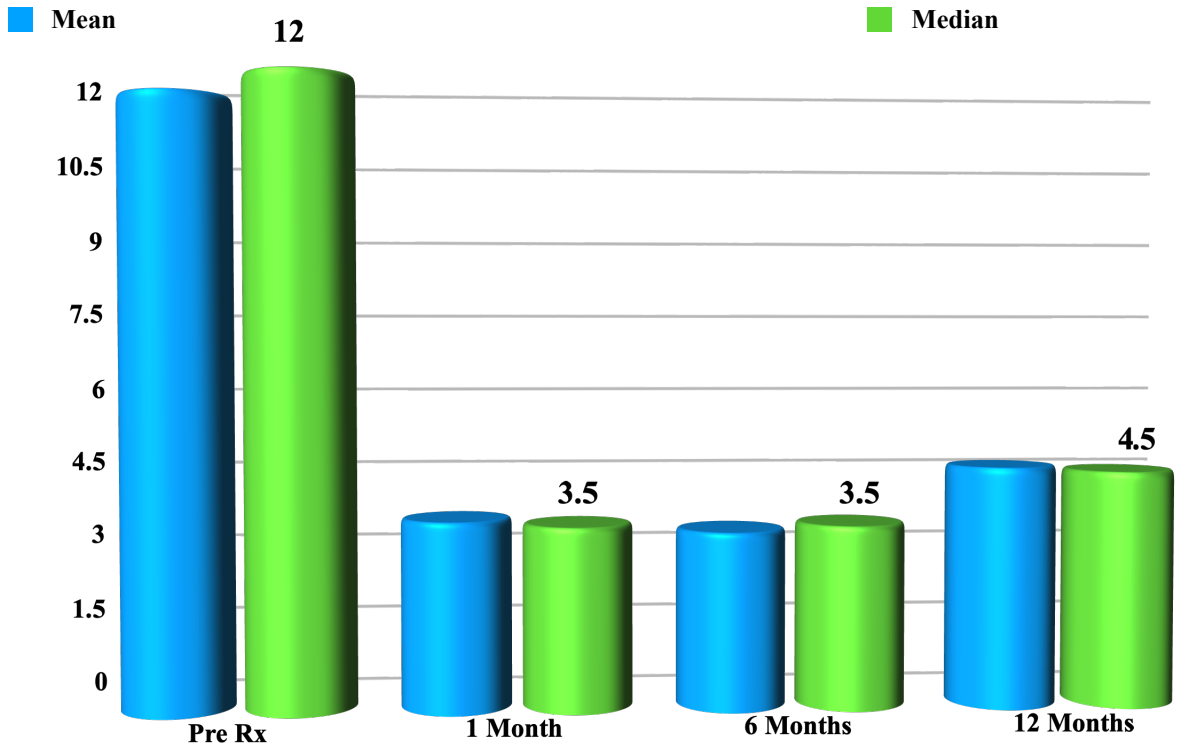


Figure : Comparison of Mean LEQ score and the Median LEQ values from Pre treatment to 12 month (n=46)

The LEQ Score; parameter for outcome progression and assessment; Pre Treatment, Mean LEQ score for study population was 11.59±3.49 with a Median of 12 (8.75-14). LEQ score at 1 month assessment were - Mean 3.61±1.49 with a Median of 3.5 (2-5)). LEQ score at 6 month assessment were - Mean 3.39±1.23 with a Median of 3.5 (2-4). LEQ score at 12 month assessment were - Mean 4.59±1.34 with a Median of 4.5 (4-6). Differences from pre treatment value found to be statistically significant.

Table : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the VAS Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46)

VAS Score	R Value	P Value
Pre Rx	.256	.086
T1	.161	.285
T6	.139	.357
T12	-.013	.934

Figure : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the VAS Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46). Observed p values do not fall within the significant limit.

Table : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Pain Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46)

WOMAC Pain Score	R Value	P Value
Pre Rx	.092	.544
T1	.099	.512
T6	.030	.844
T12	.033	.830

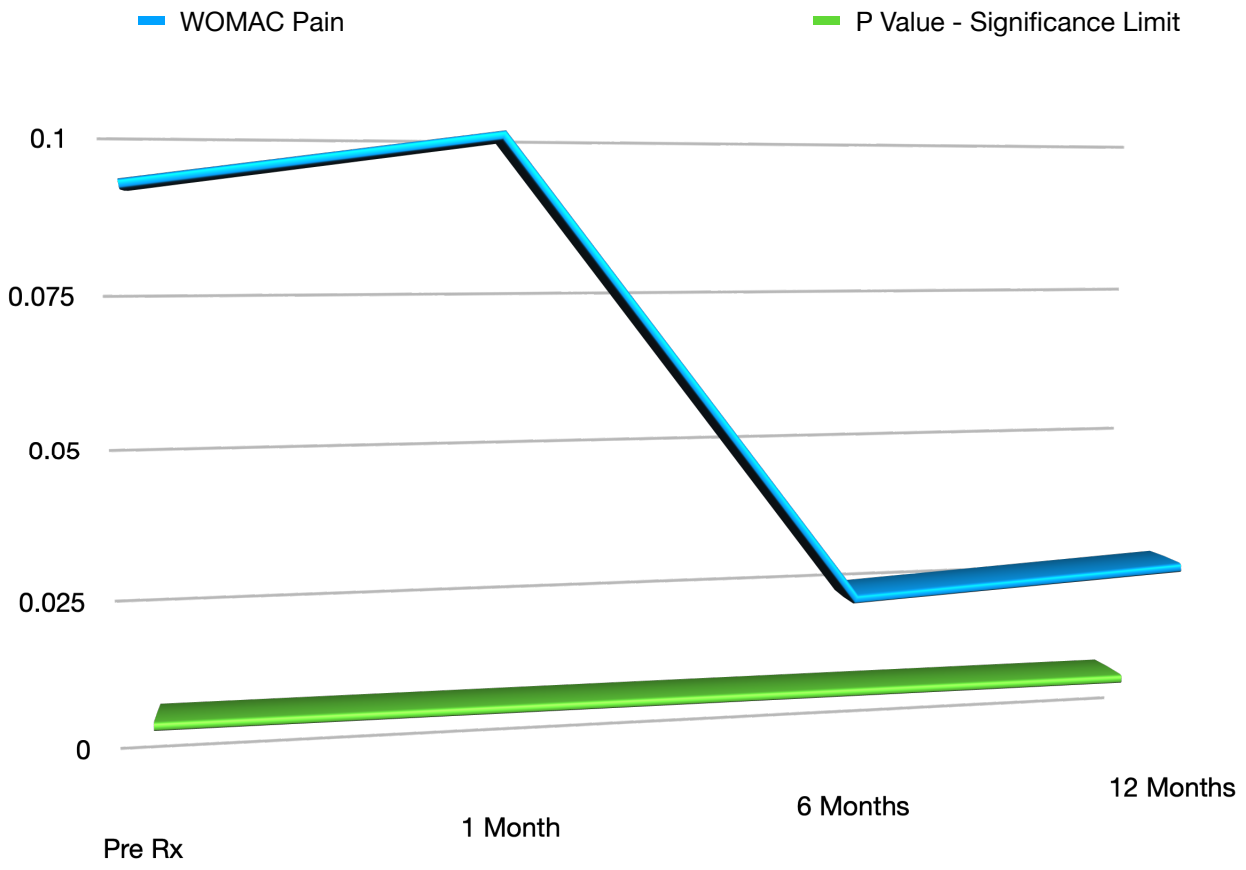


Figure : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Pain Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46). Observed p values do not fall within the significant limit.

Table : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Stiffness Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46)

WOMAC Stiffness Score	R Value	P Value
Pre Rx	.202	.178
T1	.136	.369
T6	.145	.337
T12	.107	.480

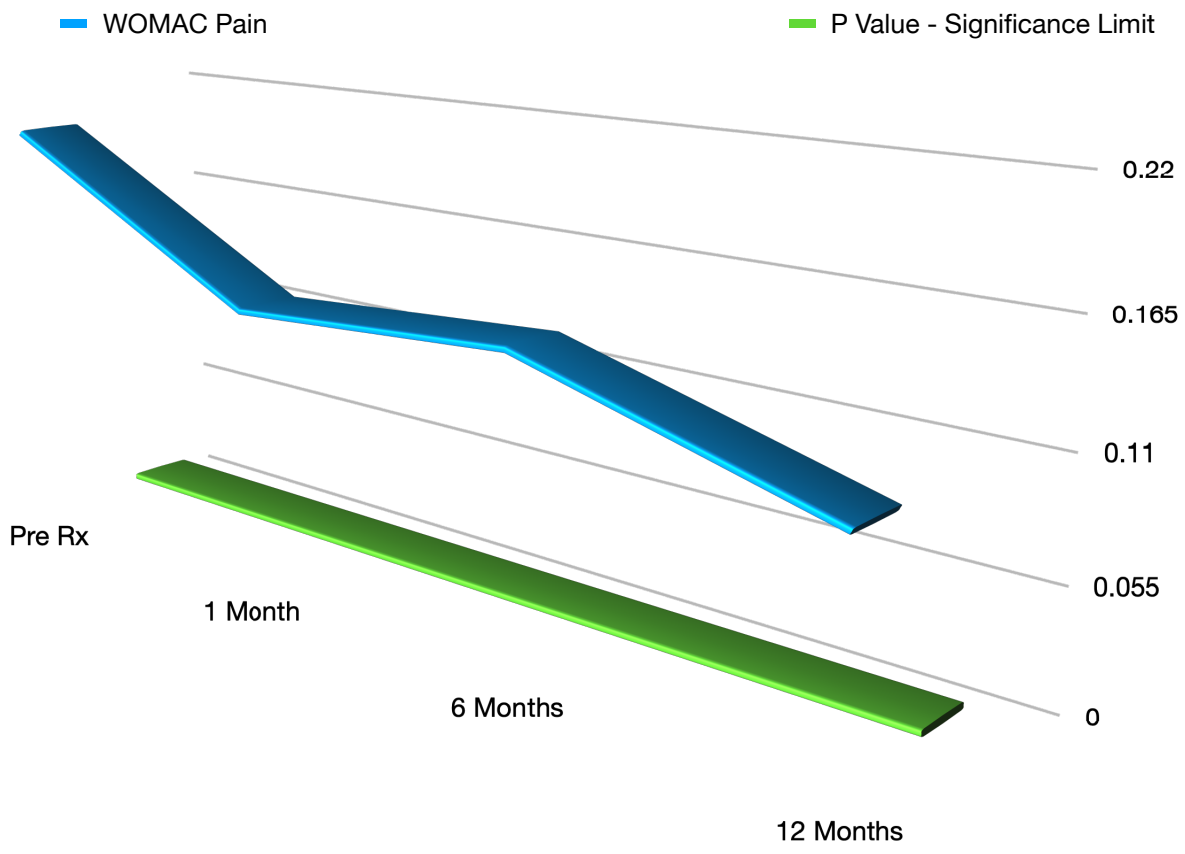


Figure : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Stiffness Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46). Observed p values do not fall within the significant limit.

Table : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Function Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46)

WOMAC Function Score	R Value	P Value
Pre Rx	.076	.618
T1	.145	.337
T6	.165	.273
T12	.123	.414

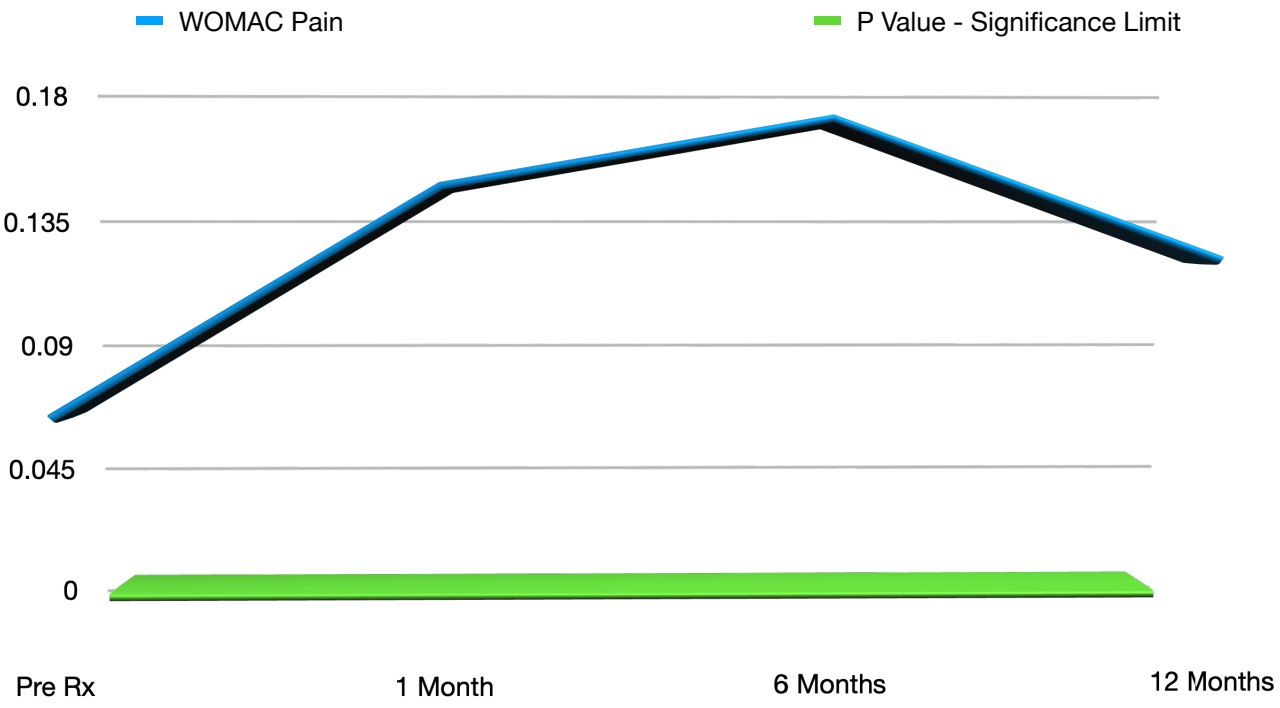


Figure : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Function Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46). Observed p values do not fall within the significant limit.

Table : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Total Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46)

WOMAC Total Score	R Value	P Value
Pre Rx	.112	.458
T1	.156	.301
T6	.152	.313
T12	.114	.452

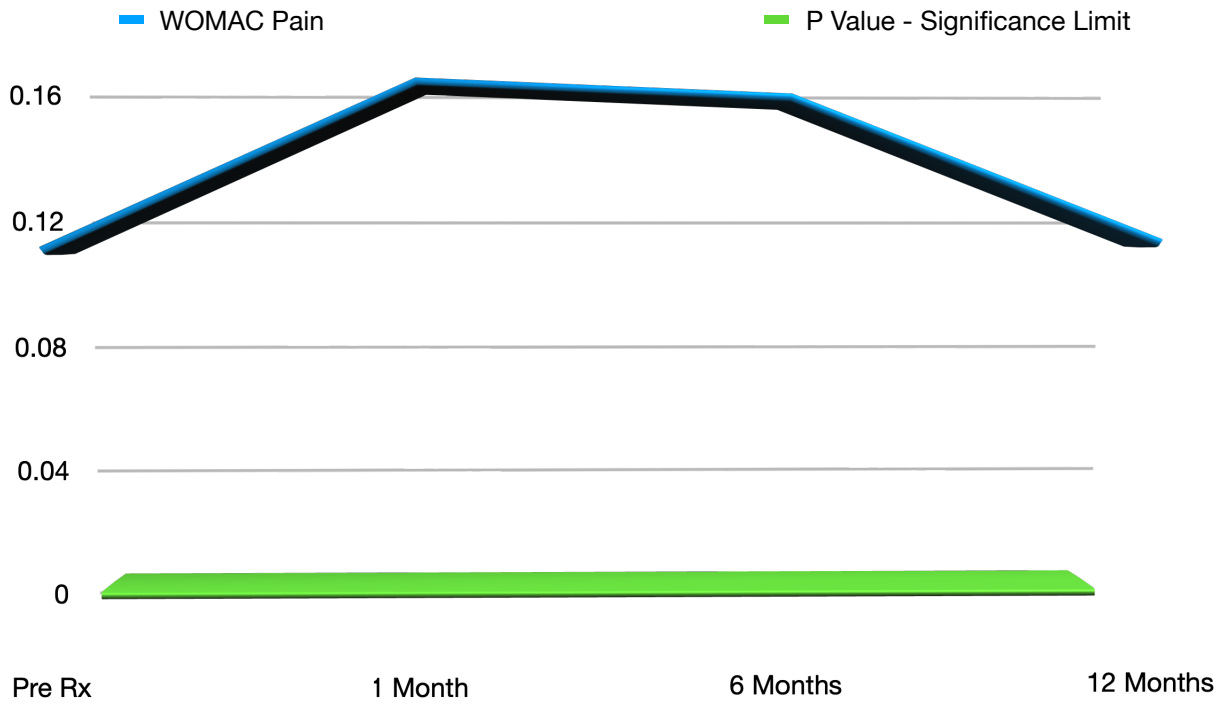


Figure : Correlations of the Kellgren-Lawrence (KL) radiologic grading with the WOMAC Total Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis (n=46. Observed p values do not fall within the significant limit.

DISCUSSION

Among the patients studied, 59% of the assessed were Female patients and 41% Male patients who did no predominantly fall within a certain age bracket but were rather well distributed along the age spectrum.

19 patients (41%) of the patients received treatment in both the knees. 12 patients (26%) and 15 patients (32%) received treatment for the Left and Right knees respectively.

83% of the patients assessed in the study had Kellegren Lawrence Grade II and early Grade III osteoarthritis of the knees and only 17 patients had Kellegren Lawrence Grade I Knee Osteoarthritis.

The assessment of the functional outcome was carried out using the Visual Analogue Scale (VAS) Score parameter; the patients presented with a mean pre treatment VAS score of 7.57±1.02 (Median score 8). The Values for the VAS Scores assessed at the 1, 6 and 12 month assessments were 2.91±1.18 (Median score 3), 1.46±0.69 (Median score 1) and 1.98±0.80 (Median score 2) respectively. The difference in the VAS Scores observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

The assessment of the functional outcome as carried out using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Score parameter was assessed in detail under the different subheadings which the score encompasses for greater statistical clarity. These Headings were composed of the WOMAC Pain Score, WOMAC Stiffness Score and the WOMAC Function Score. The WOMAC Total score could then be derived from these individual sub assessment parameters.

The assessment of the functional outcome as carried out using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Pain Score parameter presented with the following results. Patients presented with a mean pre treatment WOMAC Pain score of 15.67±2.14 (Median score 16. The Values for the WOMAC Pain assessed at the 1, 6 and 12 month assessments were 6.98±2.23 (Median score 7), 6.50±1.94 (Median score 6) and 6.89±2.01 (Median score 7) respectively. The difference in the WOMAC Pain Scores observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

The assessment of the functional outcome as carried out using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Stiffness Score parameter presented with the following results. Patients presented with a mean pre treatment WOMAC Stiffness score of 5.80±1.84 (Median score 6). The Values for the WOMAC Stiffness assessed at the 1, 6 and 12 month assessments were 3.0±1.28 (Median score 3), 2.85±1.11 (Median score 3) and 3.11±1.01 (Median score 3) respectively. The difference in the WOMAC Stiffness Scores observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

The assessment of the functional outcome as carried out using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Function Score parameter presented with the following results. Patients presented with a mean pre treatment WOMAC Function score of 53.22±8.24 (Median score 55). The Values for the WOMAC Function assessed at the 1, 6 and 12 month assessments were 19.43±4.59 (Median score 18), 18.98±4.15 (Median score 18) and 20.67±3.94 (Median score 20) respectively. The difference in the WOMAC Function Scores observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

The assessment of the functional outcome as carried out using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Total Score parameter presented with the following results. Patients presented with a mean pre treatment WOMAC Total score of 74.7±10.62 (Median score 77.5). The Values for the WOMAC Total assessed at the 1, 6 and 12 month assessments were 29.41±6.81 (Median score 28), 28.33±5.94 (Median score 27) and 30.67±5.81 (Median score 30) respectively. The difference in the WOMAC Total Scores observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

The assessment of the functional outcome as carried out using the LEQ Knee Score : Index of Severity for Osteoarthritis of the Knee (Lequesne et al.) parameter presented with the following results: Patients presented with a mean pre treatment LEQ Knee Score of 11.59±3.49 (Median score 12). The Values for the LEQ Knee Score assessed at the 1, 6 and 12 month assessments were 3.61±1.49 (Median score 3.5), 3.39±1.23 (Median score 3.5) and 4.59±1.34 (Median score 4.5) respectively. The difference in the LEQ Knee Score Scores observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

All the aforementioned assessment parameters had the following congruency in the observed statistical data :

The difference in the score values observed at 1,6 and 12 month intervals was found to be statistically highly significant, [p value < 0.001] in comparison to Pre Treatment values.

All aforementioned parameters / scores indicated maximum functionality, as present in the Knee under assessment, at the 6 month follow up interval. These objective scores and statistical data was in concurrence with the observed clinical picture at this assessment.

Subsequent to this at the 12 month follow up interval, the functionality scores in the Knee under assessment showed a mild degree of functional regression. The functional regression observed in the terms of objective scores at 12 months still remained statistically highly significant in terms of the function outcome improvement post treatment. this was in concurrence with the observable clinical assessment of the patient at these follow up intervals. These objective scores and statistical data was in concurrence with the observed clinical picture at this assessment.

Analysis to assess the correlation of the Kellgren-Lawrence (KL) radiologic grading of the affected and under assessment knee with the VAS Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis, presented with statistical values that did not underline any significant correlation to be present [p Value was not found to be within the significant range.]

The assessment of the correlation of the Kellgren-Lawrence (KL) radiologic grading of the affected and under assessment knee with the WOMAC Pain Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis, presented with statistical values that did not underline any significant correlation to be present. [p Value was not found to be within the significant range.]

The assessment of the correlation of the Kellgren-Lawrence (KL) radiologic grading of the affected and under assessment knee with the WOMAC Severity Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis, presented with statistical values that did not underline any significant correlation to be present. [p Value was not found to be within the significant range.]

The assessment of the correlation of the Kellgren-Lawrence (KL) radiologic grading of the affected and under assessment knee with the WOMAC Function Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis, presented with statistical values that did not underline any significant correlation to be present. [p Value was not found to be within the significant range.]

The assessment of the correlation of the Kellgren-Lawrence (KL) radiologic grading of the affected and under assessment knee with the WOMAC Total Score at baseline, after 1, 6 and 12 months treatment in patients with primary knee osteoarthritis, presented with statistical values that did not underline any significant correlation to be present. [p Value was not found to be within the significant range.]

SUMMARY

1. Ozone and Platelet Rich Plasma (PRP) used in a combination therapy for the treatment of primary knee osteoarthritis proves to be an effective treatment modality.

2. Ozone and Platelet Rich Plasma (PRP) used in a combination therapy for the treatment of primary knee osteoarthritis showed significantly improved Visual Analogue Scale assessment Scores indicating marked pain relief and amelioration of disease morbidity, as assessed through patient response. The data in this regard was found to be statistically highly significant.

3. Ozone and Platelet Rich Plasma (PRP) used in a combination therapy for the treatment of primary knee osteoarthritis showed significantly improved WOMAC Score across all sub parameters of Pain, Stiffness and Function indicating marked pain relief, improvement of knee function as compared to pre treatment status and amelioration of disease morbidity. The data in this regard was found to be statistically highly significant.

4. Ozone and Platelet Rich Plasma (PRP) used in a combination therapy for the treatment of primary knee osteoarthritis showed significantly improved LEQ Knee Score - Index of Severity for Osteoarthritis of the Knee (Lequesne et al.) as compared to pre treatment status indicating marked patient relief, increase in function and efficacy of treatment. The data in this regard was found to be statistically highly significant.

5. No significant correlation between the outcome and the Kellegren Lawrence degree of disease severity was established from the statistical analysis of the study data in this study.

CONCLUSION

1. Ozone and Platelet Rich Plasma (PRP) used in a combination therapy via this treatment protocol for the treatment of Kellegren Lawrence Grade 1,2 and early 3 primary knee osteoarthritis proved to be an effective treatment modality offering significant pain relief, and markedly improved function of the knee.

2. No significant correlation between the outcome and the Kellegren Lawrence degree of disease severity was established from the statistical analysis of the study data in this study.

REFERENCES

[1]Bahar Dernek, Fatma Nur Kesiktas, Istanbul Physical Therapy and Rehabilitation Application and Research Center, Saglik Bilimleri University, Istanbul, Turkey Efficacy of combined ozone and platelet-rich-plasma treatment versus platelet-rich-plasma treatment alone in early stage knee osteoarthritis. Journal of Back and Musculoskeletal Rehabilitation 32 (2019) 305–311.DOI 10.3233/BMR-181301

[2]Huang P, Wang R, Pang X, Yang Y, Guan Y, Zhang D. Platelet-rich plasma combined with ozone prevents cartilage destruction and improves weight-bearing asymmetry in a surgery-induced osteoarthritis rabbit model. Ann Palliat Med 2022;11(2):442-451. doi: 10.21037/apm-21-1510

[3]Yeprem L, Ellialtioglu A, Baeza-Noci J. The efficacy of intra-articular PRP, Ozone and Ozone+PRP injections in patients with osteoarthritis.. *J Ozone Ther.* 2018:2(3) doi: 10.7203/jo3t.2.3.2018.10904

[4] Tahir Mutlu Duymus, Serhat Mutlu, Bahar Dernek · Baran Komur, Suavi Aydogmus, Fatma Nur Kesiktas, Choice of intra-articular injection in treatment of knee osteoarthritis: platelet-rich plasma, hyaluronic acid or ozone options..Knee Surg Sports Traumatol Arthrosc DOI 10.1007/s00167-016-4110-5

[5] Lopes de Jesus CC, dos Santos FC, de Jesus LMOB, Monteiro I, Sant'Ana MSSC, Trevisani VFM (2017) Comparison between intra- articular ozone and placebo in the treatment of knee osteoarthritis: A randomized, double-blinded, placebo-controlled study. PLoS ONE 12(7): e0179185. https://doi.org/10.1371/journal. pone.0179185

[6]Arash Babaei-Ghazani ,Saeedeh Najarzadeh ,Korosh Mansoori ,Bijan Forogh ,Seyed Pezhman Madani ,Safoora Ebadi ,Hamid Reza Fadavi & Bina Eftekharsadat.The effects of ultrasound-guided corticosteroid injection compared to oxygen–ozone (O2–O3) injection in patients with knee osteoarthritis: a randomized controlled trial .International League of Associations for Rheumatology (ILAR) 2018 DOI : doi.org/10.1007/s10067-018-4147-6.

[7] Seyed Ahmad Raeissadat, Parsa Ghazi Hosseini, Mohammad Hasan Bahrami, Reza Salman Roghani, Mohammad Fathi, Azadeh Gharooee Ahangar and Mahtab Darvish.The comparison effects of intra-articular injection of Platelet Rich Plasma (PRP), Plasma Rich in Growth Factor (PRGF), Hyaluronic Acid (HA), and ozone in knee osteoarthritis; a one year randomized clinical trial..Raeissadat et al. BMC Musculoskeletal Disorders (2021) 22:134 https://doi.org/10.1186/s12891-021-04017-x

[8] Gaballa NM et al. Therapeutic efficacy of intra-articular injection of platelet–rich plasma and ozone therapy in patients with primary knee osteoarthritis. The Egyptian Rheumatologist (2018), https://doi.org/10.1016/j.ejr.2018.07.005

[9] Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Os- teoarthritis: A disease of the joint as an organ. Arthritis Rheum 2012; 64(6): 1697-707.

[10] B. *Dernek and F.N. Kesiktas / Efficacy of combined ozone and PRP treatment versus PRP treatment alone in early stage knee OA* 311

[11] Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair- Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk fac- tor clusters in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380(9859): 2224-60.

[12] Marx RE. Platelet-rich plasma (PRP): What is PRP and what is not PRP? Implant Dent 2001; 10(4): 225-8.

[13] Tayapongsak P, O'Brien DA, Monteiro CB, Arceo-Diaz LY. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. J Oral Maxillofac Surg 1994; 52(2): 161-5, (discussion 6).

[14] Raeissadat SA, Rayegani SM, Hassanabadi H, Fathi M, Ghor- bani E, Babaee M, et al. Knee osteoarthritis injection choices: Platelet-rich plasma (prp) versus hyaluronic acid (a one-year randomized clinical trial). Clin Med Insights Arthritis Muscu- loskelet Disord 2015; 8: 1-8.

[15] Rayegani SM, Raeissadat SA, Taheri MS, Babaee M, Bahrami MH, Eliaspour D, et al. Does intra articular platelet rich plasma injection improve function, pain and quality of life in patients with osteoarthritis of the knee? A randomized clinical trial. Orthop Rev (Pavia) 2014; 6(3): 5405.

[16] Sundman EA, Cole BJ, Karas V, Della Valle C, Tetreault MW, Mohammed HO, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthri- tis. Am J Sports Med 2014; 42(1): 35-41.

[17] Li JH, Zhou LX, Li GY, Cheng B. Treatment of middle-aged and aged patients with knee osteoarthritis of yang-deficiency induced cold-damp syndrome by ozone combined Chinese materia medica: A clinical research. Zhongguo Zhong Xi Yi Jie He Za Zhi 2013; 33(4): 471-5.

[18] Wang B, Dong GZ, Ju YX, Yan CS. Case-control study on therapeutic effects of ozone and triamcinolone acetonide on the treatment of meniscal injury. Zhongguo Gu Shang 2014; 27(4): 295-8.

[19] Sabanci AU, Yildiz L, Akcali DT, Karamercan A, Karamer- can A, Diracoglu D, et al. Comparison of the effects of in- traarticular ozone and intraarticular PRP in patients with os-teoarthritis. Turk J Integr Med 2015; 3(2): 31-4.

[20] Chang KV, Hung CY, Aliwarga F, Wang TG, Han DS, Chen WS. Comparative effectiveness of platelet-rich plasma injec- tions for treating knee joint cartilage degenerative pathology: A systematic review and meta-analysis. Arch Phys Med Re- habil 2014; 95(3): 562-75.

[21] Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: A health status instrument for measuring clinically important patient relevant outcomes

to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988; 15(12): 1833-40.

[22] Feng X, Beiping L. Therapeutic efficacy of ozone injection into the knee for the osteoarthritis patient along with oral cele- coxib and glucosamine. J Clin Diagn Res 2017; 11(9): UC01-

[23] Harris SE, Bonewald LF, Harris MA, Sabatini M, Dallas S, UC3.

[24] Feng JQ, et al. Effects of transforming growth factor beta on bone nodule formation and expression of bone morphogenetic protein 2, osteocalcin, osteopontin, alkaline phosphatase, and type I collagen mRNA in long-term cultures of fetal rat cal- varial osteoblasts. J Bone Miner Res 1994; 9(6): 855-63.

[25] Cardile V, Jiang X, Russo A, Casella F, Renis M, Bindoni M. Effects of ozone on some biological activities of cells *in vitro*. Cell Biol Toxicol 1995; 11(1): 11-21.

[26] Manoto S, Maepa M, Motaung S. Medical ozone therapy as a potential treatment modality for regeneration of damaged articular cartilage in osteoarthritis 2016.

[27] Al-Jaziri AA, Mahmoodi SM. Painkilling effect of ozone- oxygen injection on spine and joint osteoarthritis. Saudi Med J 2008; 29(4): 553-7.

[28] Hashemi M, Jalili P, Mennati S, Koosha A, Rohanifar R, Madadi F, et al. The effects of prolotherapy with hypertonic dextrose versus prolozone (intraarticular ozone) in patients with knee osteoarthritis. Anesth Pain Med 2015; 5(5): e27585.

[29] Lopes de Jesus CC, Dos Santos FC, de Jesus L, Mon- teiro I, Sant'Ana M, Trevisani VFM. Comparison be- tween intra-articular ozone and placebo in the treatment of knee osteoarthritis: A randomized, double-blinded, placebo- controlled study. PLoS One 2017; 12(7): e0179185.

[30] Mishra SK, Pramanik R, Das P, Das PP, Palit AK, et al. Role of intra-articular ozone in osteoarthritis of knee for func- tional and symptomatic improvement. Ind J Phys Med Reha- bilit 2011; 22(2): 65-9.

[31] Benvenuti P. Oxygen-ozone treatment of the knee, shoulder and hip: A personal experience. Rivista Italiana di Ossigeno- Ozonoterapia 2006; 5: 135-44.

[32] Giombini A, Menotti F, Di Cesare A, Giovannangeli F, Rizzo M, Moffa S, et al. Comparison between intrarticular injec- tion of hyaluronic acid, oxygen ozone, and the combination of both in the treatment of knee osteoarthrosis. J Biol Regul Homeost Agents 2016; 30(2): 621-5.

[33] Kaux JF, Croisier JL, Leonard P, Le Goff C, Crielaard JM. Exuberant inflammatory reaction as a side effect of platelet- rich plasma injection in treating one case of tendinopathy. Clin J Sport Med 2014; 24(2): 150-2.

ANNEXURE

Case History Performa

Date:

Name: Age:

Sex:

OPD Reg No: Occupation:

Address:

Chief Complaint:

H/o Present Illness:

Medical History:

Treatment/Drug history:

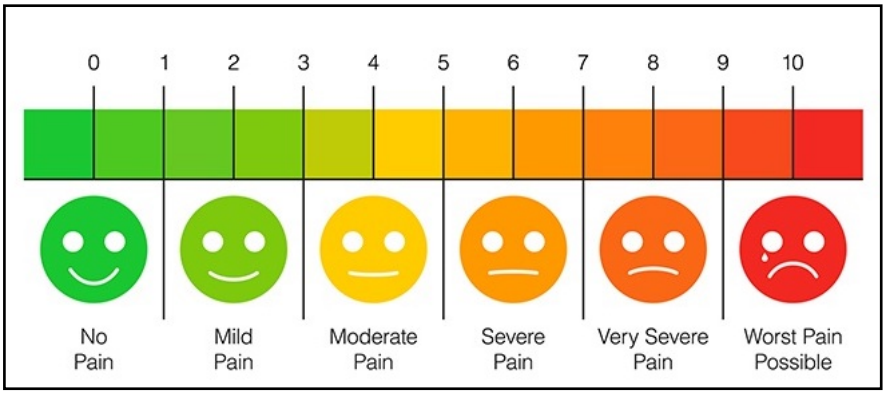
Surgical History :

Radiological Investigations:

Final Diagnosis:

Kellegren- Lawrence OA Grade :

Severity of VAS (Please mark)



SANTOSH MEDICAL COLLEGE & HOSPITAL
Department of Orthopaedics

PATIENT NAME	DOB

WESTERN ONTARIO AND MCMASTER OSTEOARTHRITIS INDEX (WOMAC)

Please circle the appropriate rating for each item.

RATE YOUR PAIN WHEN...	NONE	SLIGHT	MODERATE	SEVERE	EXTREME	HOSPITAL USE ONLY
Walking	0	1	2	3	4	
Climbing stairs	0	1	2	3	4	
Sleeping at night	0	1	2	3	4	
Resting	0	1	2	3	4	
Standing	0	1	2	3	4	TOTAL

RATE YOUR STIFFNESS IN THE...	NONE	SLIGHT	MODERATE	SEVERE	EXTREME	HOSPITAL USE ONLY
Morning	0	1	2	3	4	
Evening	0	1	2	3	4	TOTAL

RATE YOUR DIFFICULTY WHEN...	NONE	SLIGHT	MODERATE	SEVERE	EXTREME	HOSPITAL USE ONLY
Descending stairs	0	1	2	3	4	
Ascending stairs	0	1	2	3	4	
Rising from sitting	0	1	2	3	4	
Standing	0	1	2	3	4	
Bending to floor	0	1	2	3	4	
Walking on even floor	0	1	2	3	4	
Getting in/out of car	0	1	2	3	4	
Going shopping	0	1	2	3	4	
Putting on socks	0	1	2	3	4	
Rising from bed	0	1	2	3	4	
Taking off socks	0	1	2	3	4	
Lying in bed	0	1	2	3	4	
Getting in/out of bath	0	1	2	3	4	
Sitting	0	1	2	3	4	
Getting on/off toilet	0	1	2	3	4	
Doing light domestic duties (cooking, dusting)	0	1	2	3	4	
Doing heavy domestic duties (moving furniture)	0	1	2	3	4	TOTAL

PATIENT SIGNATURE	DATE

REVIEWED BY PHYSICAL THERAPIST	DATE	WOMAC TOTAL SCORE /96

WOMAC OSTEOARTHRITIS INDEX QUESTIONNAIRE

Name and signature

Thesis Study Doc No : _____

Data collected by :_____

SANTOSH MEDICAL COLLEGE & HOSPITAL
Department of Orthopaedics

Index of Severity for Osteoarthritis of the Knee (Lequesne et al.)

Patient name:.....
Date: .....

Overview: Lequesne et al developed an index of severity for osteoarthritis for the knee (ISK). This can be used to assess the effectiveness of the interventions.

Sections for index: (1) Pain or discomfort (2) Maximum distance walked (3) Activities of daily living
Your answers will enable an overall assessment of your condition over the last four weeks. Please select the box that better represent your situa

Section I: Pain or Discomfort

Parameter	Findings	Values	Points
Pain or discomfort during nocturnal bedrest (i.e. sleep)			
	None	0	
	Only on movement or in certain positions	1	
	Without movement	2	
Duration of morning stiffness or pain after getting up			
	None	0	
	< 15 minutes	1	
	>= 15 minutes	2	
Remaining standing for 30 minutes increases pain			
	No	0	
	Yes	1	
Pain on walking			
	None	0	
	Only after walking some distance	1	
	Early after starting (just when start walking)	2	
Pain or discomfort after getting up from sitting without use of arms			
	No	0	
	Yes	1	

Section II. Maximum Distance Walked

Parameter	Findings	Values	Points
Maximum distance walked			
	Unlimited	0.0	
	> 1 kilometer but limited	1.0	
	About 1 kilometer (about 15 minutes)	2.0	
	About 500 - 900 meters (about 8-15 minutes)	3.0	
	From 300 - 500 meters	4.0	
	From 100 - 300 meters	5.0	
	< 100 meters	6.0	
Walking aids required			
	None	0.0	
	One walking stick or crutch	1.0	
	Two walking sticks or crutches	2.0	

Section III. Activities of Daily Living

Parameter	Findings	Values	Points
Able to climb up a standard flight of stairs			
	Easily	0.0	
	With mild difficulty	0.5	
	With moderate difficulty	1.0	
	With marked difficulty	1.5	
	Impossible	2.0	

Parameter	Findings	Values	Points
Able to climb down a standard flight of stairs			
	Easily	0.0	
	With mild difficulty	0.5	
	With moderate difficulty	1.0	
	With marked difficulty	1.5	
	Impossible	2.0	
Parameter	**Findings**	**Values**	**Points**
Able to squat or bend at the knee			
	Easily	0.0	
	With mild difficulty	0.5	
	With moderate difficulty	1.0	
	With marked difficulty	1.5	
	Impossible	2.0	
Parameter	**Findings**	**Values**	**Points**
Able to walk on uneven ground			
	Easily	0.0	
	With mild difficulty	0.5	
	With moderate difficulty	1.0	
	With marked difficulty	1.5	
	Impossible	2.0	
Index of severity = Sum of points for all parameters			

Index of Severity for Osteoarthritis of the Knee (Lequesne et al.) Page 2 of 2

Interpretation:

- Minimum points for each section: 0
- Maximum points for each section: 8
- Minimum index score: 0
- Maximum index score: 24

Index Score	Handicap
0	None
1-4	Mild
5-7	Moderate
8-10	Severe
11-13	Very Severe
>=14	Extremely Severe

References:

Lequesne M Mery C et al. Indexes of severity for osteoarthritis of the hip and knee. Scand J Rheumatology. 1987; Supplement 65: 85-89.
Lequesne M. Indices of severity and disease activity for osteoarthritis. Seminars in Arthritis and Rheumatism. 1991; 20 (supplement 2): 48-54.
Lequesne MG. The algofunctional indices for hip and knee osteoarthritis. J Rheumatol. 1997; 24: 779-781.

Name and signature **Data collected by :**_____

Thesis Study Doc No : _____

INFORMED CONSENT

Patient Name :		**Age/ Sex**	**:**
Address	**:**	**Phone Number**	**:**
S. No	**:**	**Date**	**:**

I have been made fully aware of the details of the patient study titled **" To Assess The Functional Outcome in Patients of Osteoarthritis Knee Managed With Intra-articular Ozone and PRP Combination Therapy"** being conducted in the Department of Orthopaedics, Santosh Medical College & Hospital, Ghaziabad, U.P.

I have understood that participation in the study requires a follow up for a minimum of 1 year and I shall be expected to share personal information regarding my health etc and answer assessment questionnaires for of the same. All my questions regarding the study have been answered my satisfaction in the language understood by me.

1. I understand that I reserve the right to withdraw from the study at any given time and may choose to refuse to answer any particular question posed to me.
2. I understand that my participation in this study is strictly confidential and that no material which could identify me would be used in the analysis and in any reports based on this study.

I hereby provide my informed consent to be made a participant in the study titled **" To Assess The Functional Outcome in Patients of Osteoarthritis Knee Managed With Intra-articular Ozone and PRP Combination Therapy"** being conducted in the Department of Orthopaedics, Santosh Medical College & Hospital, Ghaziabad, U.P.

Signature / Thumb Impression of the Patient

Signature / Thumb Impression of Witness

सहभागी सुचित सहमचत प्र पत्र

गाइड का नाम :
रोगी का नाम :

आयु/लिंग :
पता :
फोन नंबर :
एसएनओ :

मुझे अध्ययन के विि रण समझाया गया है **" To Assess The Functional Outcome in Patients of Osteoarthritis Knee Managed With Intra-articular Ozone and PRP Combination Therapy"** मेरा अध्ययन के बारे में प्रश्ोोों को एक मेरे द्वारा समझा भाषा में मेरी सोंतुवि का उत्तर विया गया

1. मैं समझता हों वक मुझे वकसी भी समय अध्ययन से ि ापस लेने का अविकार है और वकसी विशेष प्रश् का उत्तर ि ेने में वगरावि आई है।

2. मैं समझता हों वक इस अध्ययन में मेरी भागी ि ारी गोपनीय है और सामग्री जो मुझे पहचान सकती है और ररपोटट इस अध्ययन के आिार पर वकसी भी में विश्लेषण उपयोग नहीों की जाएगी। मैं इस प्रकार अध्ययन में अपनी सहमवत प्रिान करता हों **" To Assess The Functional Outcome in Patients of Osteoarthritis Knee Managed With Intra-articular Ozone and PRP Combination Therapy"**

रोगी के हस्ताक्षर / अोंगूठे की छाप

गिाह के हस्ताक्षर / अोंगूठे की छाप